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Novel Organophosphorus Oligomers

Synthesis and conformation of α -hydroxy phenylphosphinates

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ABSTRACT

Chapter one reviews the recent progress in the synthesis of phosphonopeptides, pseudopeptides containing a phosphinic, phosphonic or phosphonamide linkage in place of an amide (peptide) linkage. It describes some of the general methods for the synthesis of these pseudopeptides; for example through couplings to the nitrogen of an α -aminophosphonic acid, or Michael addition to acrylates, as well as other methods, the scope for which are not as wide yet. It also provides a summary of the reported biological activities of this class of pseudopeptides.

Chapter two contains the results and discussion for a novel method for the synthesis of α -hydroxy phenylphosphinate oligomers as well as hybrid oligomers containing α -hydroxy phenylphosphinic acid and α -amino carboxylic acids. In particular, synthesis of a series of dimeric α -hydroxy phenylphosphinates are reported. The analysis of these dimers by a combination of NMR spectroscopy, X-ray crystallography and computational methods shows intramolecular hydrogen bonding in these molecules depends on the relative configuration of the carbon and phosphorus atoms. However, although the development of the synthetic methods was successful, the separation and isolation of the diastereomers was not always possible, which hindered a more comprehensive analysis of folding patterns in these molecules.

Chapter three contains the experimental procedures, preparation and spectroscopic characterisation of all the chemical compounds.

Crystal data and details of crystal structures are in the Appendix.

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ABBREVIATIONS

| | |
|-------|--|
| Å | angstrom |
| Ac | acetyl |
| ACE | angiotensin converting enzyme |
| AHEP | 1-amino-2-(4-hydroxyphenyl) ethylphosphonic acid |
| APP | amyloid precursor protein |
| app. | apparent |
| atm. | atmosphere |
| aq. | aqueous |
| BACE1 | human β -secretase |
| Bn | benzyl |
| BOC | <i>t</i> -butoxycarbonyl |
| BOP | benzotriazole-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate |
| BSA | bis(trimethylsilyl)acetamide |
| BTSA | bis(trimethylsilyl)amine |
| Bu | butyl |
| Bz | benzoyl |
| Cal B | <i>Candida antarctica</i> lipase B |
| cat. | catalyst |
| cbz | carbobenzyloxy / benzyloxycarbonyl |
| cod | 1,5-cyclooctadiene |
| conc. | concentrated |

| | |
|------------------|---|
| CPA | carboxypeptidase A |
| CSA | camphor-10-sulfonic acid |
| DBU | 1,8-diazabicyclo[5.4.0]undec7-ene |
| DCC | <i>N,N'</i> -dicyclohexylcarbodiimide |
| DDQ | 2,3-dichloro-5,6-dicyanobenzoquinone |
| DEAD | diethyl azodicarboxylate |
| DIC | 1,3-diisopropylcarbodiimide |
| dioxane | [1,4]-dioxane |
| DIPEA | diisopropylethylamine |
| DMAP | 4-dimethylaminopyridine |
| DMF | <i>N,N</i> -dimethylformamide |
| DMSO | dimethylsulfoxide |
| DPP IV | dipeptidyl peptidase IV |
| EDCI | 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide |
| Et | ethyl |
| eq. | equivalent |
| Fmoc | 9-fluorenylmethoxycarbonyl |
| HBTU | <i>O</i> -benzotriazole- <i>N,N,N',N'</i> -tetramethyl-uronium-hexafluoro-phosphate |
| HIV-1 | human immunodeficiency virus 1 |
| HMDS | hexamethyldisilazane |
| HOBt | 1-Hydroxybenzotriazole |
| HOESY | heteronuclear overhauser enhancement spectroscopy |
| HPLC | high performance liquid chromatography |
| IC ₅₀ | Half maximal inhibitory concentration |

| | |
|------------------------|---|
| Kcal | kilocalorie |
| lit. | literature |
| <i>m</i> | <i>meta</i> |
| Me | methyl |
| MMP | matrix metalloproteinase |
| Ms | methanesulfonyl (mesyl) |
| MW | microwave |
| <i>p</i> -NA | <i>p</i> -nitroaniline |
| NAPAP | N ^α -(2-naphthyl-sulfonyl-glycyl)-DL- <i>p</i> -amidinophenylalanyl-piperidine |
| NEM | <i>N</i> -ethylmorpholinium acetate |
| nm | nanometer |
| NMP | <i>N</i> -methylpyrrolidine |
| NMR | nuclear magnetic resonance |
| NOESY | nuclear overhauser enhancement spectroscopy |
| <i>o</i> | <i>ortho</i> |
| <i>p</i> | <i>para</i> |
| PDT | photodynamic therapy |
| Ph | phenyl |
| PMB | <i>p</i> -methoxybenzyl |
| ppm | parts per million |
| ^{<i>i</i>} Pr | isopropyl |
| py | pyridine |
| PyPOP | (pentafluorophenyl) oxytripyrrolidinophosphonium hexafluorophosphate |
| R _f | retention factor |

| | |
|-------|---|
| rt | room temperature |
| SrtA | <i>Staphylococcus aureus</i> sortase transpeptidase |
| TBAF | tetra- <i>n</i> -butyl ammonium fluoride |
| TBDMS | <i>t</i> -butyldimethylsilyl |
| TBTU | <i>O</i> -benzotriazole-1-yl-1,1,3,3-tetramethyluronium tetrafluoroborate |
| TFA | trifluoroacetic acid |
| THF | tetrahydrofuran |
| TLC | thin layer chromatography |
| TMS | trimethylsilyl |
| TMSBr | bromotrimethylsilane |
| TMSCl | trimethylsilylchloride / chlorotrimethylsilane |
| TMSI | iodotrimethylsilane |
| Trt | triphenylmethyl (trityl) |
| Ts | 4-methylbenzenesulfonyl (tosyl) |
| UA | Ursolic acid |
| uPA | urokinase plasminogen activator |

1. Chapter One: Recent advances in the chemistry of phosphonopeptides

1.1. Introduction

Phosphonopeptides are structural analogues of peptides in which one or more of the amide functions have been replaced by a phosphono function. A variety of different phosphonopeptides exist depending on the phosphono group. The phosphorus atom may be bonded to a nitrogen atom, an oxygen atom or a carbon atom (**Figure 1**).

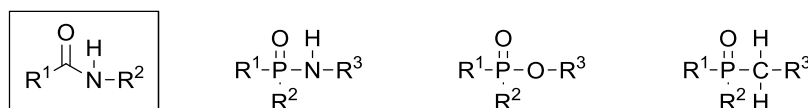


Figure 1 Comparison of amide and phosphono groups.

Recent years have seen an increasing interest in synthetic and naturally occurring phosphonopeptides as a significant class of *peptidomimetics* in medicinal and bioorganic chemistry.^{1,2,3} Phosphonopeptides are useful in developing peptidase enzyme inhibitors, since the phosphono functions may be construed as transition state analogs of the scissile amide bond (**Figure 2**)^{4,5,6} as well as haptens for producing catalytic antibodies with esterase activity.^{7,8} Many phosphonopeptides have been shown to be useful as anticancer, antibiotic and antibacterial agents.^{9,10} In addition, over the last decade, a number of phosphonopeptides with α -aminophosphonic acid at the C terminus have been synthesised as herbicides.¹¹

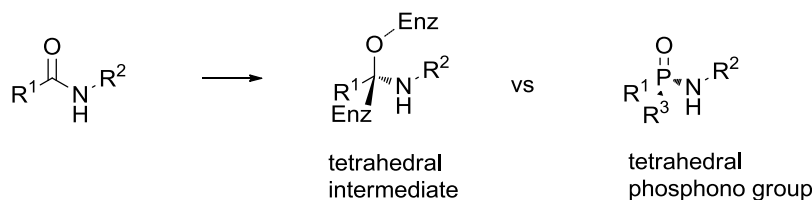


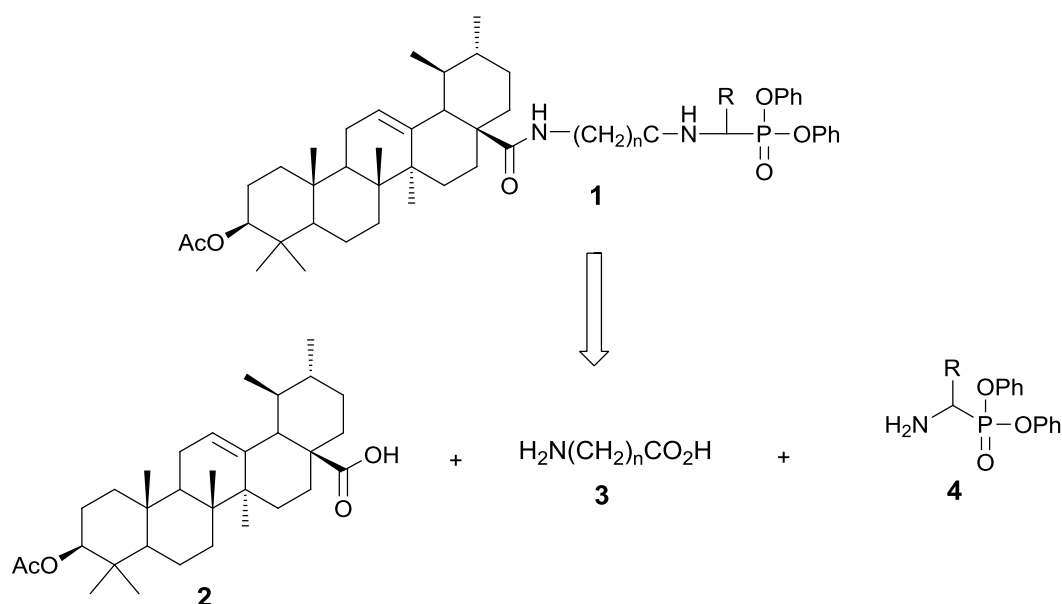
Figure 2 Comparison of amide-enzyme intermediate and tetrahedral phosphono group.

This review covers the recent reports of the preparation of phosphonopeptides. Synthesis of phosphonopeptides has been comprehensively reviewed previously^{12,13} and older synthetic approaches to them can be ascertained from the references cited there as

well as the body of this review. Phosphopeptides, peptides that contain one or more phosphate groups, resulting from phosphorylation of the hydroxyl function in tyrosine or serine residues, will not be covered in this review as their chemistry and application are considerably different.

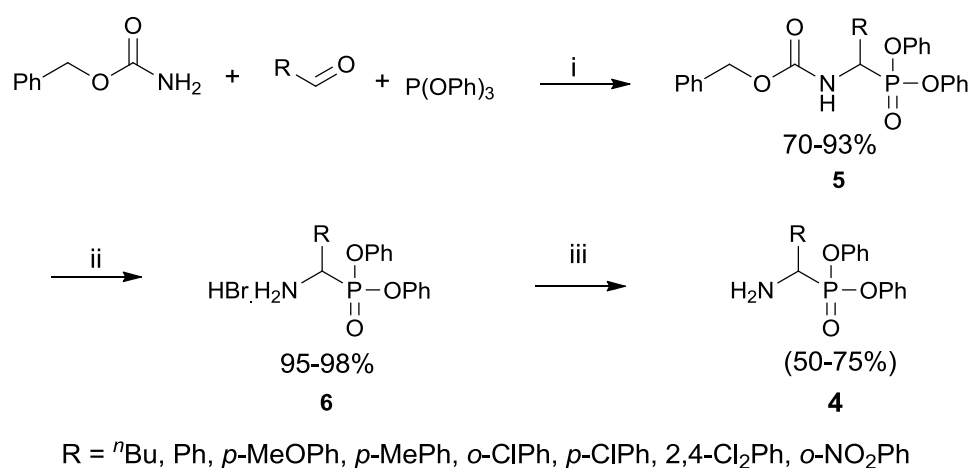
1.2. Reaction involving coupling of an α -aminophosphonate and an acid

One of the most common routes to phosphonopeptides is to ligate an existing α -aminophosphonic moiety to the carboxy terminus of a peptide. There are already a large number of methods for the synthesis of α -aminophosphonates (some of which will also be shown here for the sake of completeness) as well as for the formation of peptide bonds. Therefore, this method has the dual advantage of being both general and reliable. In line with a similar methodology in peptide chemistry, coupling of an α -aminophosphonate with the acid chloride of a suitably protected α -amino carboxylic acid is a straight forward route to a phosphonopeptide. The method, which is exemplified shortly, obviously requires synthesis of an α -aminophosphonic acid to start with, but it also suffers in that acid chlorides of suitably protected α -amino carboxylic acids are difficult to produce with chiral integrity.



Scheme 1 Retrosynthetic analysis of ursolic acid phosphonopeptide derivative.

Deng *et al.*¹⁴ synthesised a series of novel phosphonopeptide derivatives of naturally occurring 3 β -hydroxyurs-12-en-28-oic acid (ursolic acid, UA) (**1a-j**) (**Scheme 4**), and have reported on their broad spectrum of biological activity. The retrosynthetic analysis for formation of the phosphonopeptides is outline in **Scheme 1**. Ursolic acid has been shown to be a potential chemotherapeutic and chemopreventive agent^{15,16,17,18} with some derivatives showing potential activity against human immunodeficiency virus 1 (HIV-1).

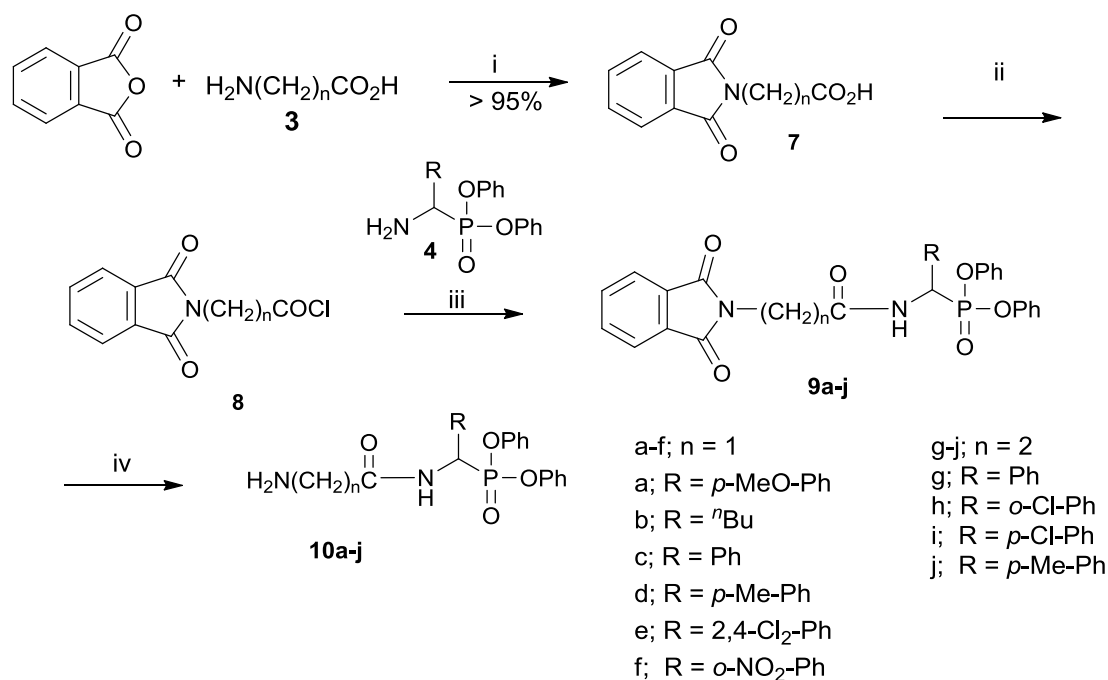


Scheme 2 Reagents and conditions: (i) AcOH, 80-85 °C, 2 h; (ii) HBr/AcOH, rt, 2 h; (iii) Et₃N/THF.

The synthesis was divided into three parts. First a number of α -aminophosphonates were synthesised using a three-component Mannich type reaction as the key step (**Scheme 2**).

Secondly, phosphonodipeptides and their homologs were synthesised through coupling of the α -aminophosphonates with an acid chloride of protected glycine or β -alanine (**Scheme 3**). Finally, phosphonodipeptide conjugates of ursolic acid and their homologs were synthesised by condensation of the chloride of UA with the phosphonodipeptides and their homologs (**Scheme 4**).

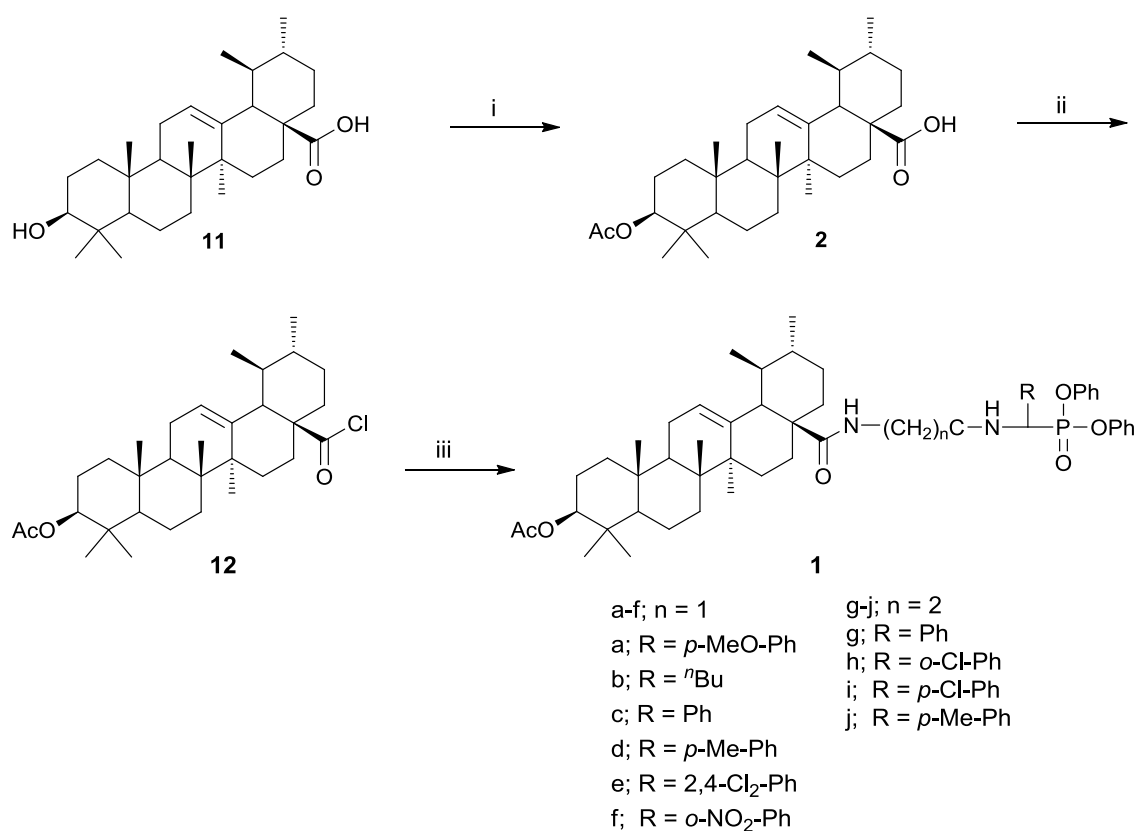
The α -aminophosphonates were synthesised according to **Scheme 2**. A one-pot, three component (benzyl carbamate, various aldehydes and triphenylphosphite) reaction was used to obtain a varied range of N-protected α -aminophosphonates, **5**. Deprotection was carried out using hydrogen bromide and acetic acid to give compounds, **6** which were then treated with triethylamine in THF to afford the desired α -aminophosphonates **4**.



Scheme 3 Reagents and conditions: (i) 185-200 °C, 15 min; (ii) SOCl₂, 60 °C, 3 h; (iii) 2 eq. Et₃N/THF, 0 °C, 15 min; then rt, overnight; (iv) NH₂NH₂·H₂O, EtOH, rt, 16 h.

The synthesis of the phosphonodipeptides and homologs was carried out as outlined in **Scheme 3**. Condensing the α -amino acids with phthalic anhydride afforded **7**. The N-protected amino acid was chlorinated using thionyl chloride to afford acid chloride **8**. The acid chloride **8** was coupled with α -aminophosphonate **4** (previously synthesised) using triethylamine to give the protected phosphonodipeptide **9**. Hydrazine was used to

remove the phthalyl protecting group to give the desired phosphonodipeptide and homolog **10**. Finally, new phosphonodipeptide conjugates of ursolic acid were synthesised according to **Scheme 4**. To form the phosphorus conjugates of UA **1**, it first had to be appropriately protected. The hydroxyl group in ursolic acid **11** was converted to an acetate, **2**, which was then chlorinated using thionyl chloride to give the corresponding acid chloride **12**. Phosphonodipeptide **10** was condensed with acid chloride **12** to give the final compound **1**.



Scheme 4 Reagents and conditions: (i) Ac_2O , DMAP, py, rt, 2.5 h; (ii) SOCl_2 , 65 °C, 5 h; (iii) **10a-j**, $\text{Et}_3\text{N/THF}$, 0 °C, 0.5 h; then rt, overnight.

An alternative, less forcing method, is to convert the carboxylic acid to a mixed anhydride *via* treatment with a chloroformate. This protocol was used by Li *et al.*¹⁹ for the synthesis of novel thrombin inhibitors containing phosphinic peptide mimetics through structure-based design. Thrombin is the final protease involved in a complex process that leads to blood coagulation; therefore its role in thrombosis and haemostasis

is significant. The inhibitors synthesised in the work are based on the structure of N^α-(2-naphthyl-sulfonyl-glycyl)-DL-*p*-amidinophenylalanyl-piperidine (NAPAP) (**Figure 3**) which has been shown to be a potent antithrombotic.

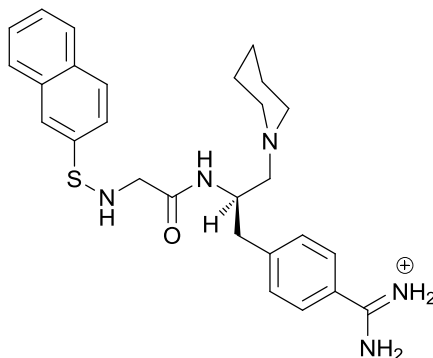
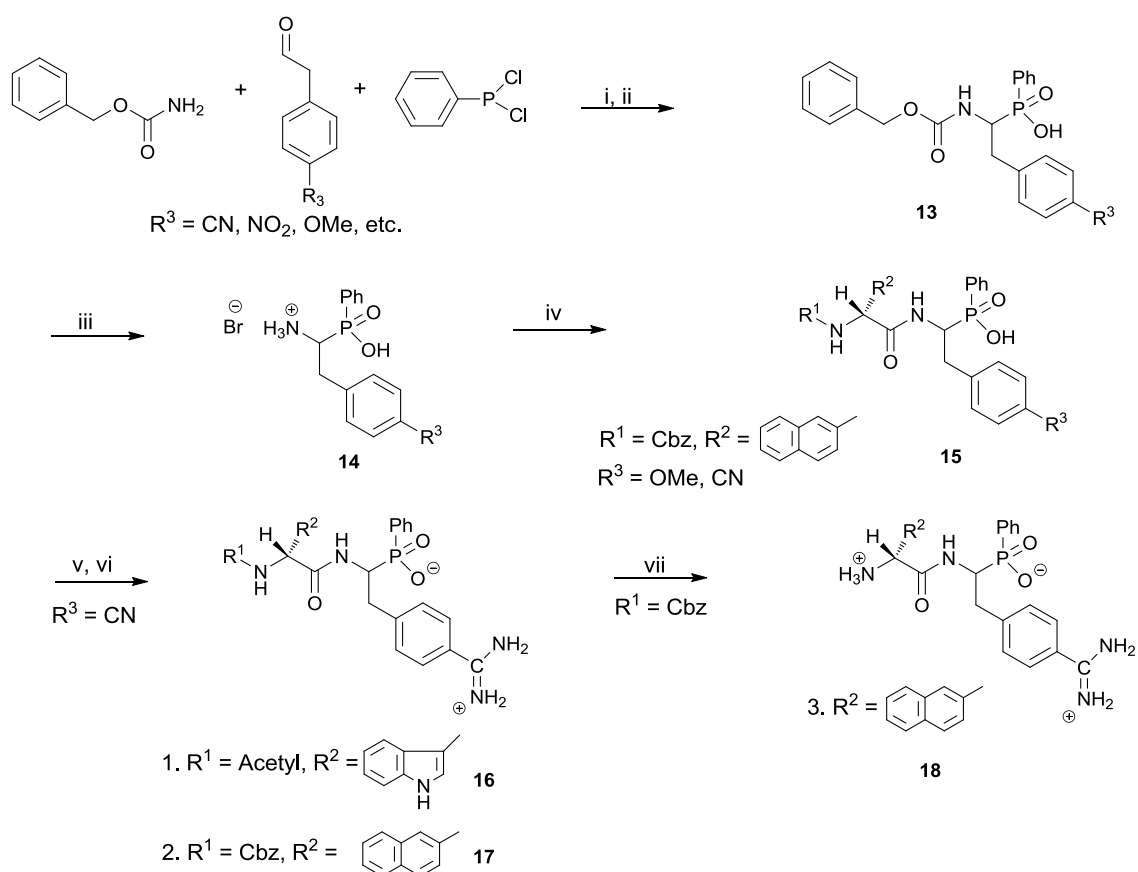


Figure 3 Structure of NAPAP.



Scheme 5 Reagents and conditions: (i) AcOH, rt, overnight; (ii) H₂O, 60%; (iii) 30% HBr/AcOH, 1 h, rt, 83-91%; (iv) R¹NHCHR²CO₂H, EtOCOC₂H₅, Et₃N, THF, -4 °C, 1 h; then rt, 24 h, 23-40%; (v) HCl/CHCl₃/MeOH, 0-4 °C, 2 d; (vi) NH₃/MeOH, rt, 2 d; (vii) 30% HBr/AcOH, rt, 1 h, 90%.

The syntheses of the phosphinic peptide mimics are carried out as shown in **Scheme 5**. The Cbz-protected phosphinic amino acids **13** were synthesised in a one pot reaction of 4-substituted phenylacetaldehydes with dichlorophenylphosphine and benzyl carbamate in acetic acid (also see **Scheme 48**).

Deprotection of **13** using hydrogen bromide in acetic acid afforded compounds **14**, which were then coupled with the N-protected amino acids that had been activated with ethyl chloroformate to give the mixed anhydrides, affording the dipeptide mimetics **15**. It is worth noting that the hydroxyl group of the phosphate does not undergo the coupling reaction. The final step involved conversion of the cyano group of **15** into an amidino group to give compounds **16** and **17**. Compound **17** required further deprotection with hydrogen bromide in acetic acid to afford **18**.

Once the inhibitors had been synthesised they were evaluated for potency in thrombin inhibition by use of a chromogenic substrate S-2238, monitoring the release of nitrophenol at 405 nm. Based on the inhibition data (**Table 1**) it could be concluded replacement of the indole ring by the 2-naphthyl ring improved activity. The increased potency of **18** compared to **17** can be explained by the deblocking of the N-terminus of **17** that could give favourable electrostatic interactions with the side chain due to the positive charge on the nitrogen.

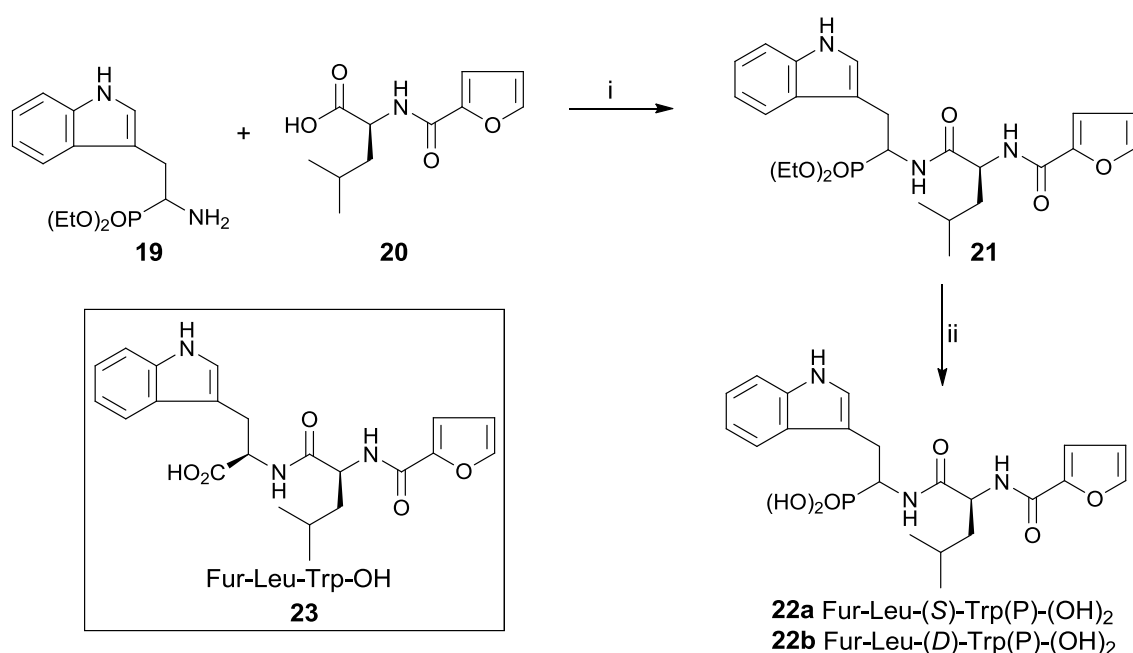
| Inhibitor | IC ₅₀ (μM) |
|-----------|-----------------------|
| 22 | 70 |
| 23 | 7.5 |
| 24 | 3.6 |
| 25 | 0.6 |

Table 1 *In vitro* inhibition by phosphinic dipeptide mimetics.

The chloroformate approach was also used by D'Alessio *et al.*²⁰ in the synthesis of phosphonate analogues of snake venom peptides as inhibitors of adamalysin II and matrix metalloproteases (MMPs). In this investigation, phosphonate analogues of the

peptidomimetic *N*-(furan-2-yl)carbonyl-Leu-Trp-OH (Fur-Leu-Trp-OH) were synthesised for evaluation of the effect of replacing a phosphonate for carboxylate in binding with MMPs (see also **Scheme 7**).

The synthesis of phosphonate analogues **22a-b** (**Scheme 6**) began by coupling **19** with **20** using a chloroformate activation to give **21**. Compound **21** was subsequently deprotected to give the phosphonate analogues of Fur-Leu-Trp-OH **22a-b**. The synthesised compounds were tested as adamalysin and MMPs 2, 3, 8 and 9 inhibitors (**Table 2**).

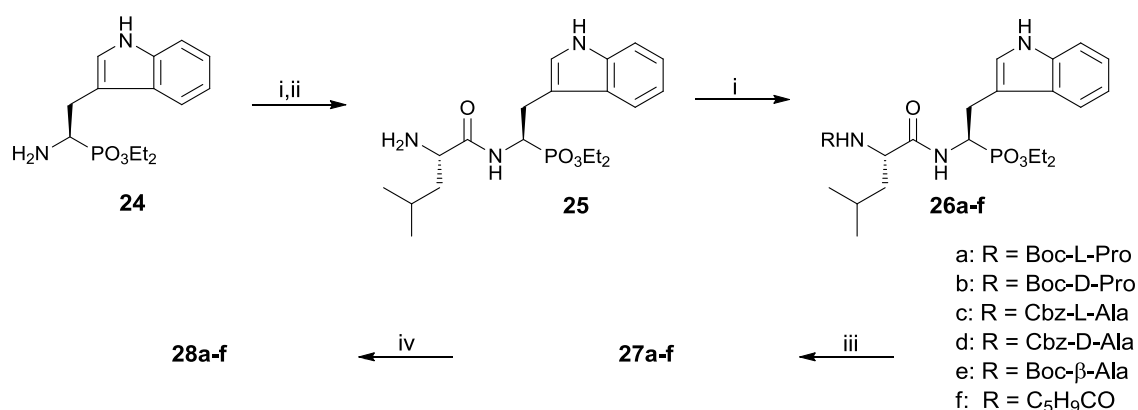


Scheme 6 Reagents and conditions: (i) *i*butyl chloroformate, *N*-methylmorpholine, 4 °C, 12 h, 65%; (ii) *N,O*-bis-trimethylsilyl-trifluoroacetamide, TMSI, CH₂Cl₂, 25 °C, 2 h, 79-82%.

| No. | Inhibitor | IC ₅₀ (μM) | | | | |
|------------|---|-----------------------|-------|-------|-------|-------|
| | | adamalysin | MMP-2 | MMP-9 | MMP-8 | MMP-3 |
| 22a | Fur-Leu-(<i>S</i>)-Trp(P)-(OH) ₂ | 0.4 | 60 | - | >100 | - |
| 22b | Fur-Leu-(<i>R</i>)-Trp(P)-(OH) ₂ | 70 | - | - | - | - |
| 23 | Fur-Leu-Trp-OH | 30 | 40 | >100 | 50 | >100 |

Table 2 *In vitro* inhibition of adamalysin and MMPs 2, 3, 8 and 9 by phosphonate inhibitors.

Of course, the most common reagent for the formation of peptide bonds is *N,N'*-dicyclohexylcarbodiimide (DCC) and its analogues. In further continuation of their earlier report (**Scheme 6**), Agamennone *et al.*²¹ synthesised and evaluated a number of tripeptide phosphonate inhibitors against MMP-8 and MMP-2, using DCC methodology (**Scheme 7**). Their work relates to synthesis of analogues of phosphotryptophan, specifically its derivative L-Pro-L-Leu-L-(P)Trp(OH)₂ a left-hand-side phosphonate inhibitor of MMP-8.



Scheme 7 *Reagents and conditions:* (i) R-AA-OH, DCC, HOBT, THF, 80-90%; (ii) 10% Pd/C, HCO₂NH₄, CH₃OH, 92%; (iii) TMSI, BSA, CH₂Cl₂, 49-92%; (iv) Ac₂O, py, 70-90%; (**26c** and **26d** require hydrogenolysis (ii)).

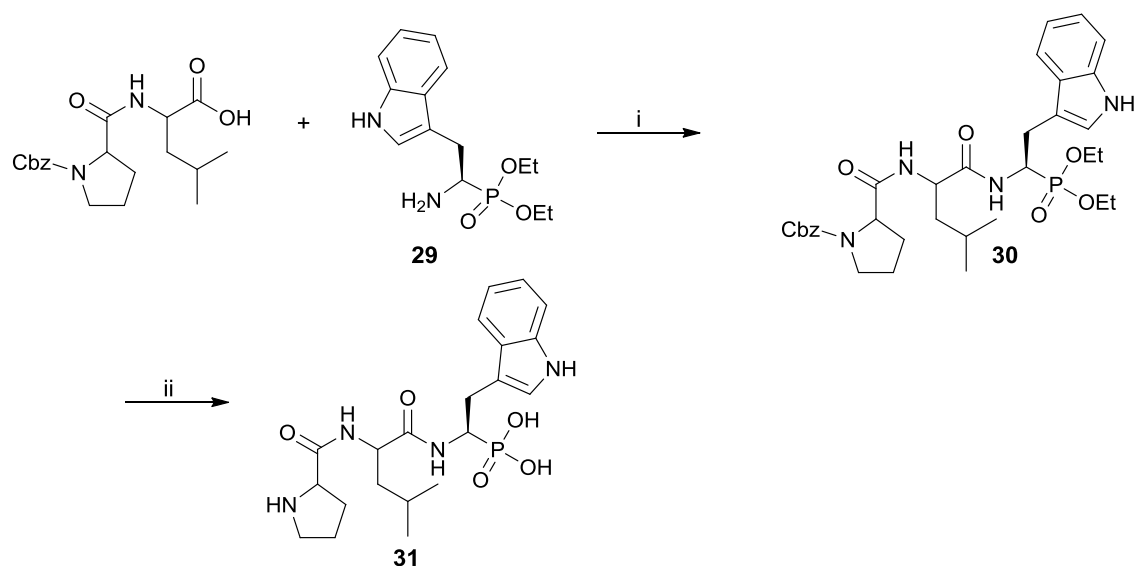
Phosphonopeptide **24** was coupled with a protected amino acid (e.g. leucine) to give **25**, followed by further coupling with a protected amino acid to give **26a-f**. Deprotection of **26a-f** gave **27a-f** which was subsequently protected with acetic anhydride to give **28a-f**. The synthesised analogues were tested for activity against MMP-2 and MMP-8 (**Table 3**). The analogues showed varied selectivity for the MMPs tested. One issue the group reported is the fact the analogues were accommodated in the right-hand side of the MMP-8 active site instead of the left as had been predicted.

| Phosphonate | | MMP-2 | MMP-8 |
|-------------|----------------------------------|---------------------|---------------------|
| No. | R | K _i (mM) | K _i (mM) |
| 27a | L-Pro | 1.53 | 3.94 |
| 27b | D-Pro | - | - |
| 27c | L-Ala | 0.4 | 0.5 |
| 27d | D-Ala | - | 0.41 |
| 27e | β-Ala | - | 0.34 |
| 27f | C ₅ H ₉ CO | 0.4 | 1.36 |
| 28a | N-Ac-L-Pro | 1.76 | 0.34 |
| 28b | N-Ac-D-Pro | - | - |
| 28c | N-Ac-L-Ala | 0.55 | 0.26 |
| 28d | N-Ac-D-Ala | 3.77 | 2.95 |
| 28e | N-Ac-β-Ala | 0.56 | 0.49 |

Table 3 Inhibition of MMP 2 and 8 by phosphonate inhibitors.

Gavuzzo *et al.*²² have used phosphinic peptidomimetics to obtain transition state analogues that are capable of interacting on both sides of the catalytic zinc ion in MMPs. The group had taken evidence of complexing the peptidase astacin with a phosphinic inhibitor²³ and substitution at P1' and P2 positions in phosphinic pseudopeptides²⁴ to design inhibitors to selectively block particular MMPs.

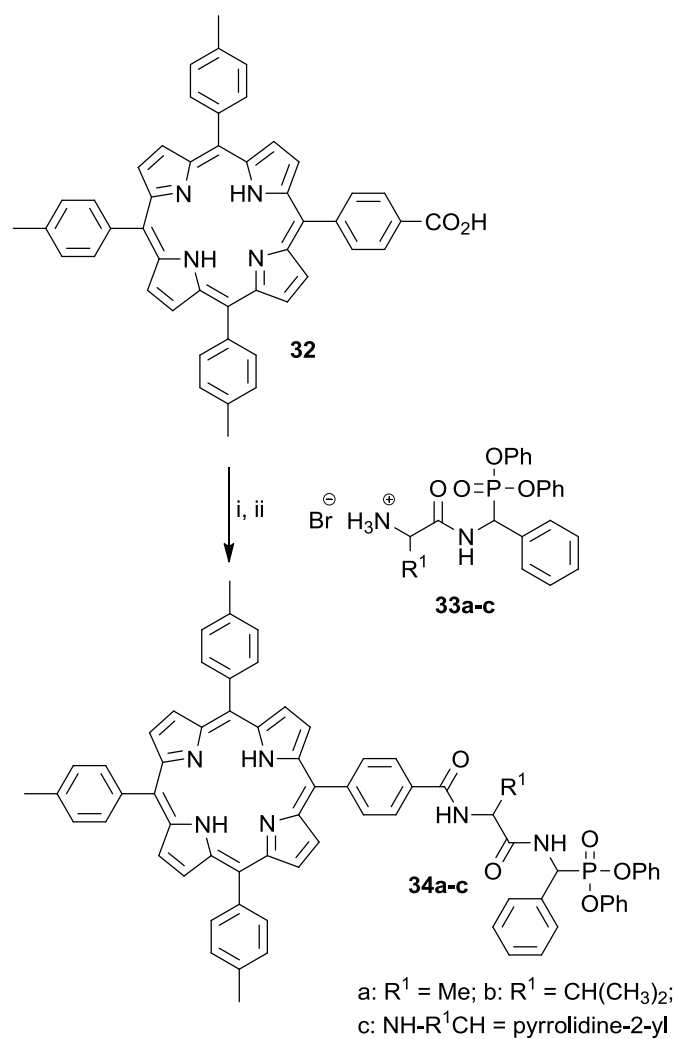
The group attempted to synthesise a number of phosphorus containing peptidomimetics based on data obtained from the isolation of phosphoramidon, a natural inhibitor of thermolysin and other metalloproteinases. The work involves the synthesis of tripeptide phosphonates as shown in **Scheme 8**



Scheme 8 Reagents and conditions: (i) DCC, HOBt, THF; (ii) TMSI, BSA, CH₂Cl₂.

This synthesis follows peptide coupling conditions where Cbz-Pro-Leu-OH was coupled with the diethyl ester of (*S*)-phosphotryptophan **29** using DCC and 1-hydroxybenzotriazole (HOBt) to give **30**. The desired phosphonate **31** was obtained by deprotecting **30** using iodotrimethylsilane (TMSI) in the presence of bistrimethylsilylacetamide (BSA) to hydrolyse the diethyl phosphonate and also remove the carbobenzyloxy (Cbz) group to give **31**.

Habdas *et al.*²⁵ synthesised three new porphyrin-peptidyl-phosphonate derivatives (**Scheme 9**) as potential photosensitisers for the photodynamic therapy (PDT) of tumours. The compounds were tested for activity as inhibitors of aminopeptidase N (**Table 4**) and showed moderate inhibition.



Scheme 9 Reagents and conditions: (i) Et_3N ; (ii) DCC, CH_2Cl_2 .

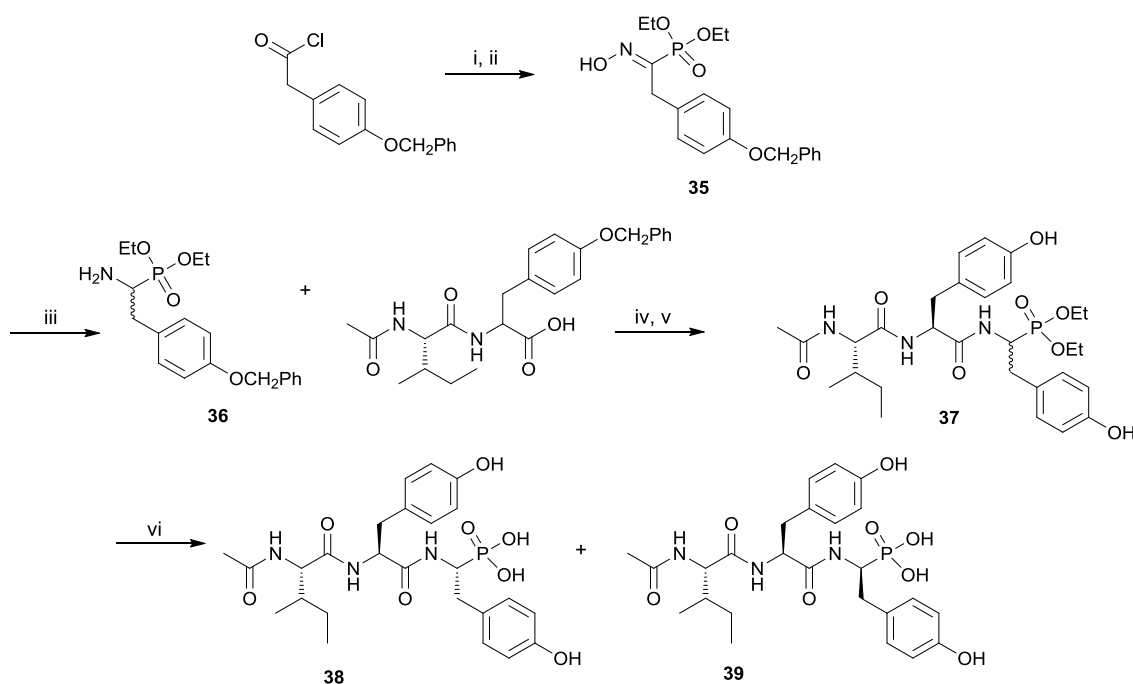
During the synthesis of the compounds they coupled the carboxylic acid of porphyrin **32** with the ammonium bromide of phosphinic peptide **33a-c** to give porphyrin-peptidyl-phosphonate derivatives **34a-c**.

| No. | Name | Mw | IC_{50} (μM) |
|------------|-------------------|------|------------------------------------|
| 32 | TTP-COOH | 701 | 18.5 |
| 34a | TTP-Ala-3PyP(OPh) | 1094 | 87.3 |
| 34b | TTP-Val-3PyP(OPh) | 1122 | 19.3 |
| 34c | TTP-Pro-3PyP(OPh) | 1120 | 32.4 |

Table 4 Inhibition of aminopeptidase N by phosphonate derivatives.

In addition to DCC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) has also found wide spread application in the formation of peptide bonds in general, and in the

context of the phosphonopeptide synthesis. The EDCI reagent was used by Ntai *et al.*²⁶ as part of their investigation to identify the pharmacophore of the angiotensin converting enzyme (ACE) in the naturally occurring phosphonotripeptide K-26, a potent inhibitor of that enzyme. The group used the protocol to synthesise (**Scheme 10**) and test eight analogues of K-26, in order to gain information into the structure activity relationships of K-26.



Scheme 10 Reagents and conditions: (i) $\text{P}(\text{OEt})_3$, THF 0 °C to 70 °C; (ii) $\text{NH}_2\text{OH}\cdot\text{HCl}$, py, EtOH, 25 °C; (iii) Zn/HCOOH , 25 °C; (iv) $\text{EDC}\cdot\text{HCl}$, HOBt , 2,4,6-trimethylpyridine, DMF, 25 °C; (v) H_2 , Pd/C , 25 °C; (vi) TMSI, thioanisole, MeCN, 0 °C.

Compound 35 was synthesised through an Arbuzov type reaction by reacting 4-benzyloxyphenylacetyl chloride with triethyl phosphite giving the corresponding α -ketophosphonate, which was converted to oxime 35 by treatment with hydroxylamine. Compound 35 was reduced with zinc and formic acid to give the racemic 1-amino-2-(4-hydroxyphenyl) ethylphosphonic acid (AHEP) diethyl ester 36. Coupling of the amine of 36 with a dipeptide, followed by hydrogenation of the benzyl protecting groups gave 37. Removal of the ethyl groups by treatment of TMSI afforded a mixture of the two major diastereomers of K-26 (38 and 39), that could be separated by chromatography.

After synthesis of the compounds, measurement of ACE activity was carried out. The IC_{50} values obtained are shown in the following table (**Table 5**).

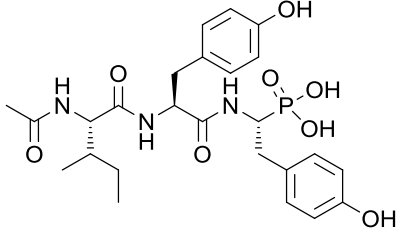
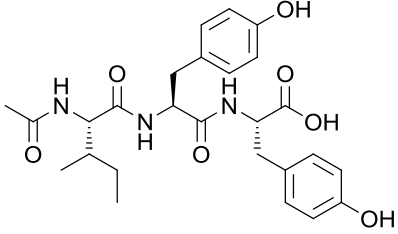
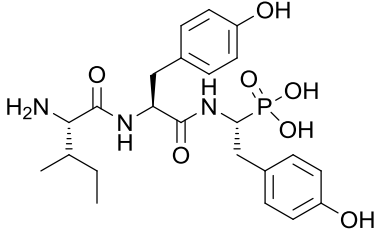
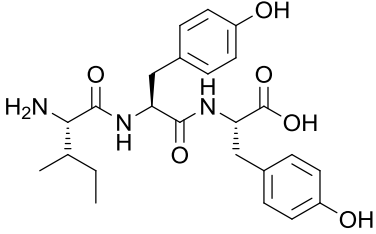
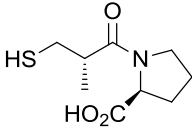
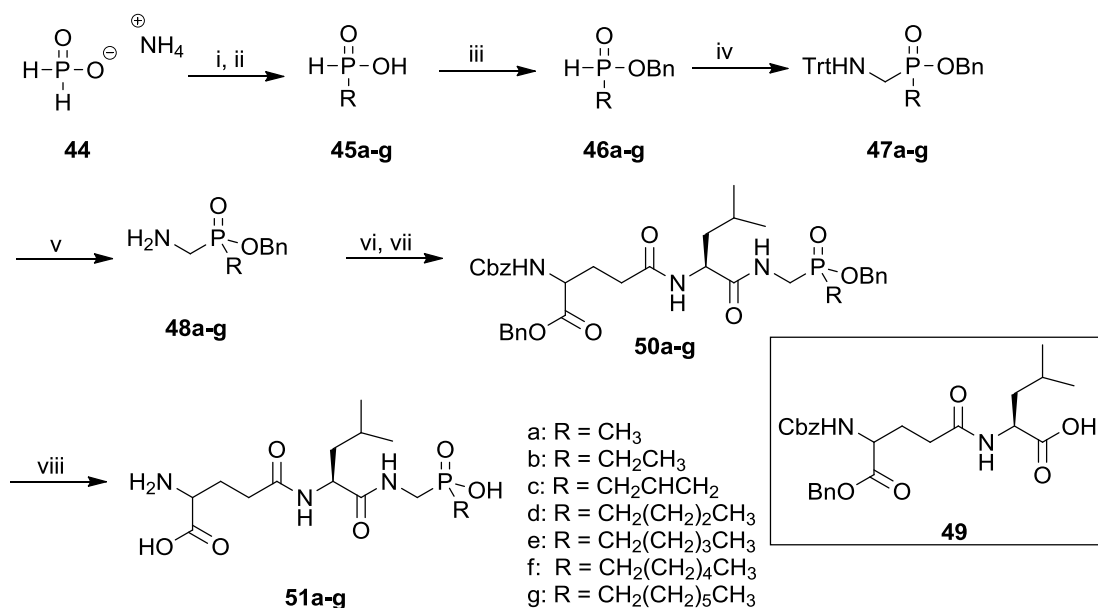
| No. | IC_{50} (nM) | No. | IC_{50} (nM) |
|---|--------------------|---|--------------------|
|  | |  | |
| 38 (K-26) Ac-(L)-Ile-(L)-Tyr-(R)-AHEP | 14.4 | 42a Ac-(L)-Ile-(L)-Tyr-(L)-Tyr | 2.1×10^4 |
| 39 Ac-(L)-Ile-(L)-Tyr-(S)-AHEP | 139 | 42b Ac-(L)-Ile-(L)-Tyr-(D)-Tyr | 2.0×10^6 |
| 40 Ac-(L)-Ile-(L)-Tyr-(R)-AHEP (OEt) ₂ | 3.83×10^5 | | |
|  | |  | |
| 41a (L)-Ile-(L)-Tyr-(S)-AHEP | 234.3 | 43a (L)-Ile-(L)-Tyr-(L)-Tyr | 2.36×10^5 |
| 41b (L)-Ile-(L)-Tyr-(R)-AHEP | 5.46×10^4 | 43b (L)-Ile-(L)-Tyr-(D)-Ty | $>10^6$ |
|  | | | |
| Captopril | 7.7 | | |

Table 5 Inhibition of ACE by K-26 analogues.

As can be seen N-acetylation of the natural peptide analogues **43a-b** to give **42a-b** increased the activity 10-fold. The introduction of the phosphonyl substitution **38** gave 1500-fold more potent activity compared to the natural tripeptide analogue **42a**.

Ravashcino *et al.*²⁷ synthesised a number of phosphinopeptides (**Scheme 11**) for use against *Trypanosoma cruzi* (Chagas's disease).



Scheme 11 *Reagents and conditions:* (i) HMDS, 110 °C, 2 h, (ii) RI, CH₂Cl₂, rt, 16 h; (iii) BnOH, DCC, DMAP, THF, rt, 16 h; (iv) TrtN=CH₂, BF₃·Et₂O, toluene, rt, 3 d; (v) TFA (2.5%), CH₂Cl₂, rt, 30 min; (vi) *N*-hydroxysuccinimide, EDC.HCl, DMF, rt, 16 h, (vii) Et₃N, DMF, rt, 16 h; (viii) H₂, Pd/C, MeOH, 1 atm, rt, 1 h.

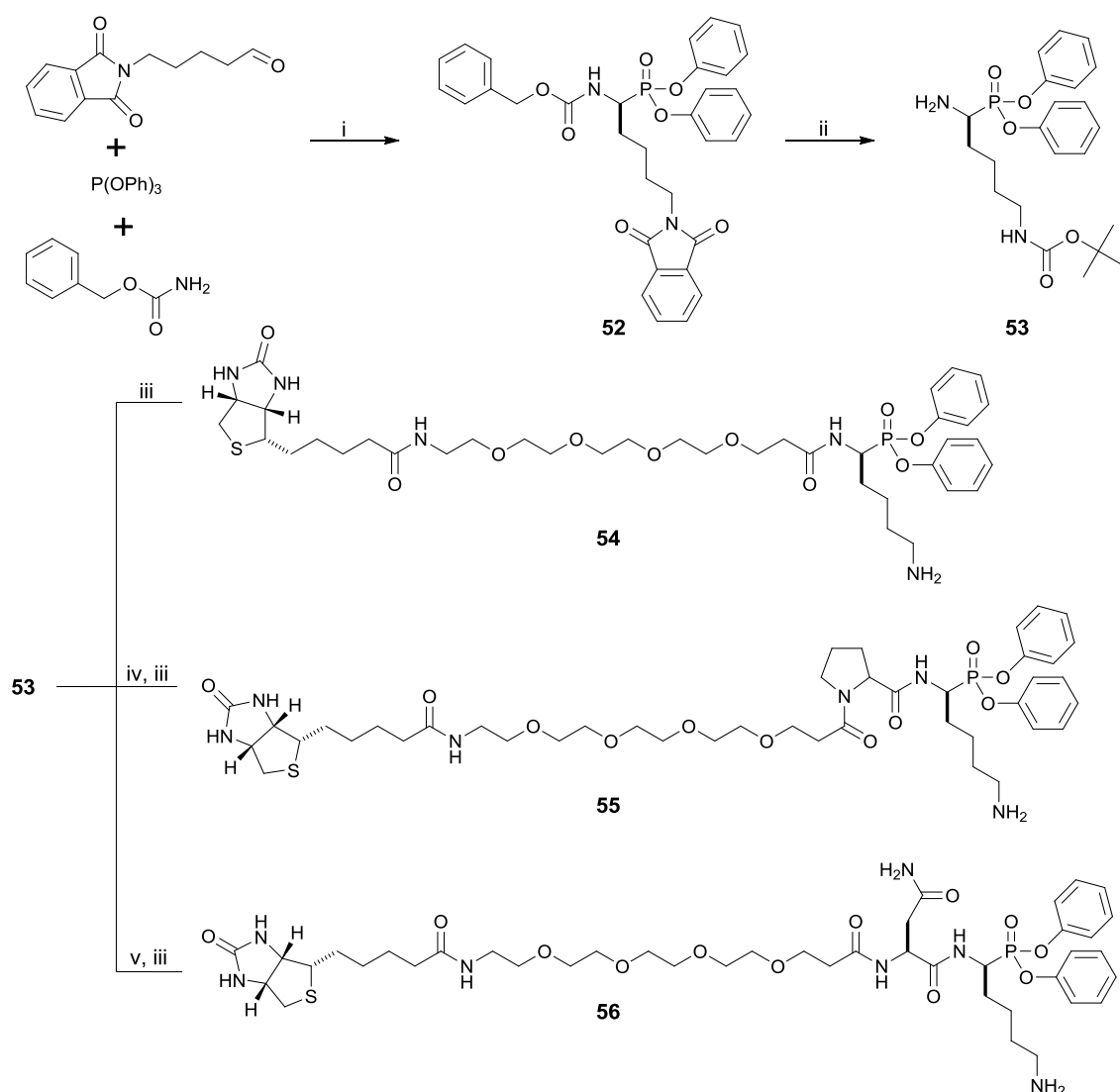
| No. | amastigotes IC ₅₀ (μM) | epimastigotes IC ₅₀ (μM) |
|-----|--------------------------------------|--|
| 51a | >50 | >50 |
| 51b | >50 | >50 |
| 51c | >50 | >50 |
| 51d | 9.8 | >50 |
| 51e | 14.2 | >50 |
| 51f | >50 | >50 |
| 51g | >50 | >50 |

Table 6 Effect of phosphinopeptides against *Trypanosoma cruzi*.

Ammonium hypophosphite **44** was activated using hexamethyldisilazane (HMDS) then coupled with various alkyl chains to give **45**. Benzylation of the alcohol in **45** was carried out to give **46**, which was then reacted with the imine to give **47**. The trityl protecting group in **47** was removed to give **48**. Coupling of **48** to dipeptide **49** afforded **50**, which was subsequently deprotected using hydrogenolysis to give **51** (Scheme 11).

After compounds **51a-g** were synthesised they were evaluated for activity against amastigotes and epimastigotes (**Table 6**).

Pan *et al.*²⁸ synthesised a series of diphenylphosphonates as activity-based probes for trypsin-family serine proteases. The work was aimed at synthesising probes that could specifically target serine proteases.

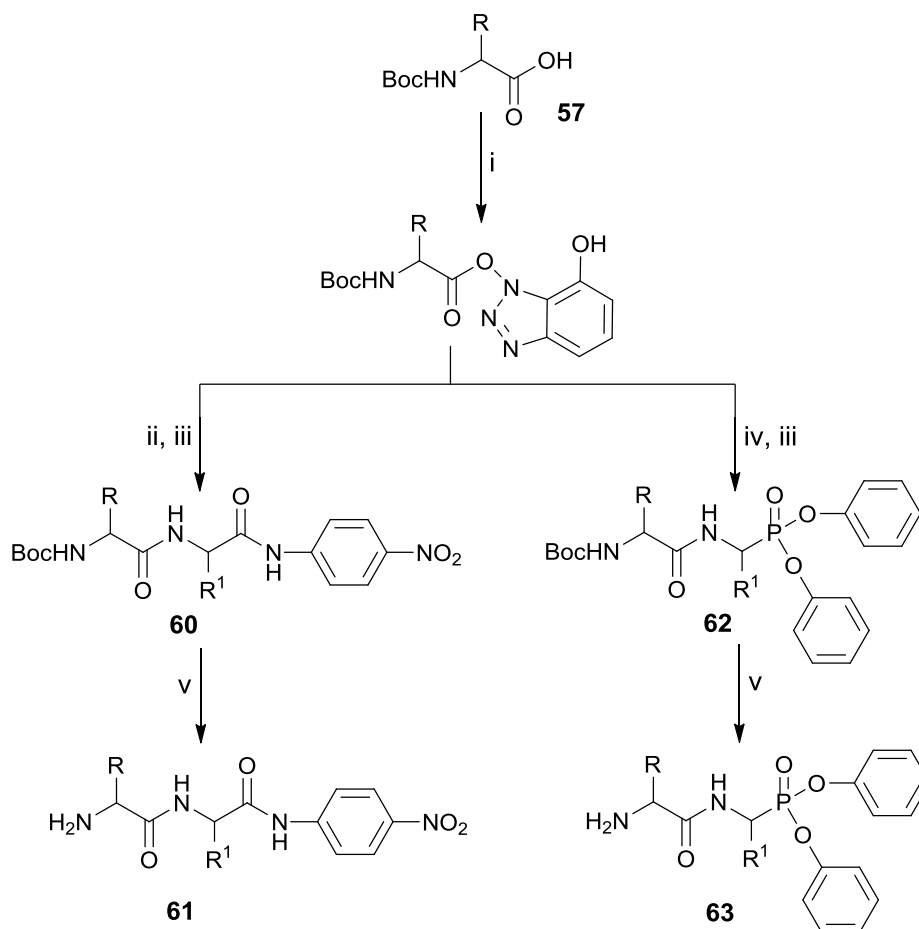


Scheme 12 Reagents and conditions: (i) AcOH, MW 150 °C, 5-10 min / oil bath 70 °C, 1-3 h; (ii) NH_2NH_2 ; Boc_2O ; H_2 , Pd/C; (iii) NHS-(PEG)₄-biotin, Et_3N ; then TFA; (iv) Cbz-Pro-OH, EDCI, HOBT; (v) Cbz-Asn(Trt)-OH, EDCI, HOBT; H_2 , Pd/C.

The synthesis of the activity based probes (**Scheme 12**) involves a three component reaction, with an amine, aldehyde and triphenylphosphite to give **52**. Subsequent

deprotection of **52** using hydrazine to remove the phthalimide group followed by *t*-butoxy carbonyl (Boc) protection, and hydrogenation of the Cbz group to give **53**. Compound **53** was then coupled through varied methods to give **54-56**.

Senten *et al.*²⁹ synthesised dipeptide *p*-nitroanilides and dipeptide diphenyl phosphonates using polymer supported carbodiimide for a solution-phase parallel synthesis (**Scheme 13**).



Scheme 13 Reagents and conditions: (i) Polymer bound carbodiimide, HOBT; (ii) R¹aa-*p*NA **58**; (iii) Polymer bound polyamine; (iv) R¹aa^P(OPh)₂ **59**; (v) TFA, CH₂Cl₂.

Boc protected amino acids **57** were activated as their 3-hydroxybenzotriazole (HOBt) esters using polymer bound carbodiimide followed by reactions with either a *p*-nitroanilide (*p*-NA) of amino acids **58** or an α -amino phosphonate **59** to give **60** and **62**. The final step involved deprotection of **60** and **62** to give dipeptide *p*-nitroanilides **61**

and dipeptide diphenyl phosphonates **63**. The full details for the dipeptides synthesised is given in the following tables (**Table 7** and **Table 8**).

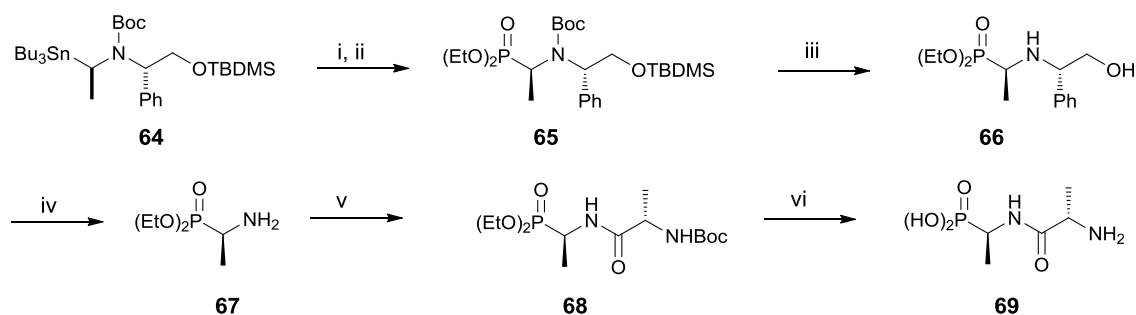
| | Pro- <i>p</i> -NA | | | Ala- <i>p</i> -NA | | |
|-----|-------------------|-------------|-----------|-------------------|-------------|-----------|
| Raa | Purity (60) | Purity (61) | Yield (%) | Purity (60) | Purity (61) | Yield (%) |
| Asn | 99 | 94 | 98 | | | |
| Asp | 90 | 95 | 87 | | | |
| Gly | | | | 97 | 97 | 86 |
| His | 40 | 91 | 42 | 97 | 96 | 49 |
| Ile | 88 | 98 | 10 | | | |
| Phe | 97 | 99 | 16 | 99 | 95 | 95 |
| Pro | 96 | 99 | 47 | 98 | 97 | 94 |
| Ser | 84 | 90 | 84 | | | |
| Tyr | 91 | 95 | 18 | 98 | 94 | 75 |
| Val | 88 | 92 | 30 | | | |

Table 7 Synthesised dipeptide *p*-nitroanilides.

| | Pro ^P (OPh) ₂ | | | Ala ^P (OPh) ₂ | | |
|---------|-------------------------------------|-------------|-----------|-------------------------------------|-------------|-----------|
| Raa | Purity (62) | Purity (63) | Yield (%) | Purity (62) | Purity (63) | Yield (%) |
| Ala | 58 | 96 | 36 | 76 | 91 | 51 |
| Asn | 87 | 99 | 59 | 80 | 77 | 23 |
| Asp | 66 | 98 | 48 | 53 | 91 | 32 |
| Gly | 64 | 94 | 61 | 78 | 94 | 60 |
| His | 31 | 100 | 7 | 30 | 100 | 27 |
| Ile | | | | 85 | 97 | 21 |
| Lys | 66 | 100 | 51 | | | |
| Phe | 83 | 95 | 58 | 89 | 97 | 13 |
| Pro | | | | 90 | 95 | 55 |
| Ser | 68 | 84 | 60 | 56 | 83 | 18 |
| ThiaPro | 75 | 95 | 41 | | | |
| Tyr | | | | 90 | 95 | 65 |
| Val | 69 | 90 | 41 | 94 | 95 | 13 |

Table 8 Synthesised dipeptide diphenyl phosphonates.

In addition to carbodiimide reagents such as DCC and EDCI, a number of other peptide coupling agents are also used.

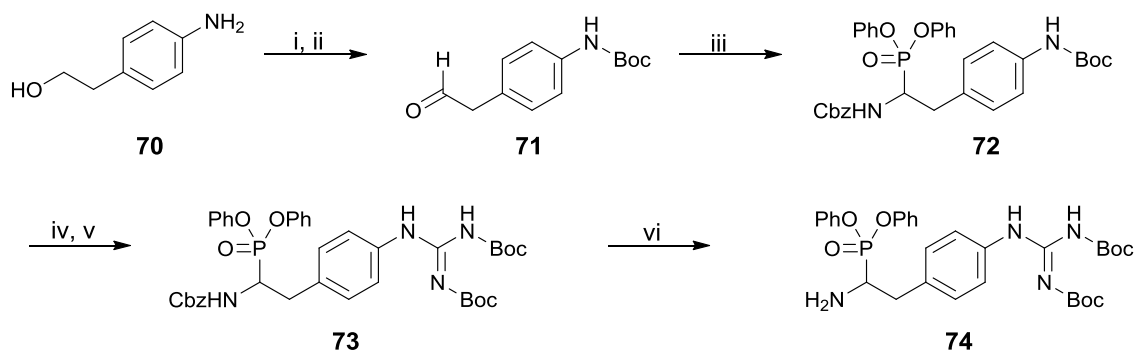


Scheme 14 Reagents and conditions: (i) $n\text{BuLi}$, THF; (ii) Diethyl chlorophosphate; (iii) TFA, CH_2Cl_2 , 67%; (iv) H_2 , $\text{Pd}(\text{OH})_2$, EtOH; (v) Boc-(L)-Ala, BOP, Et_3N , CH_2Cl_2 , 79%; (vi) HBr/AcOH.

Coeffard *et al.*³⁰ report the synthesis of alafosfalin **69** using a methodology they developed involving the use of enantioenriched tributylstannylated α -amino alcohols and a benzotriazole-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) coupling protocol.

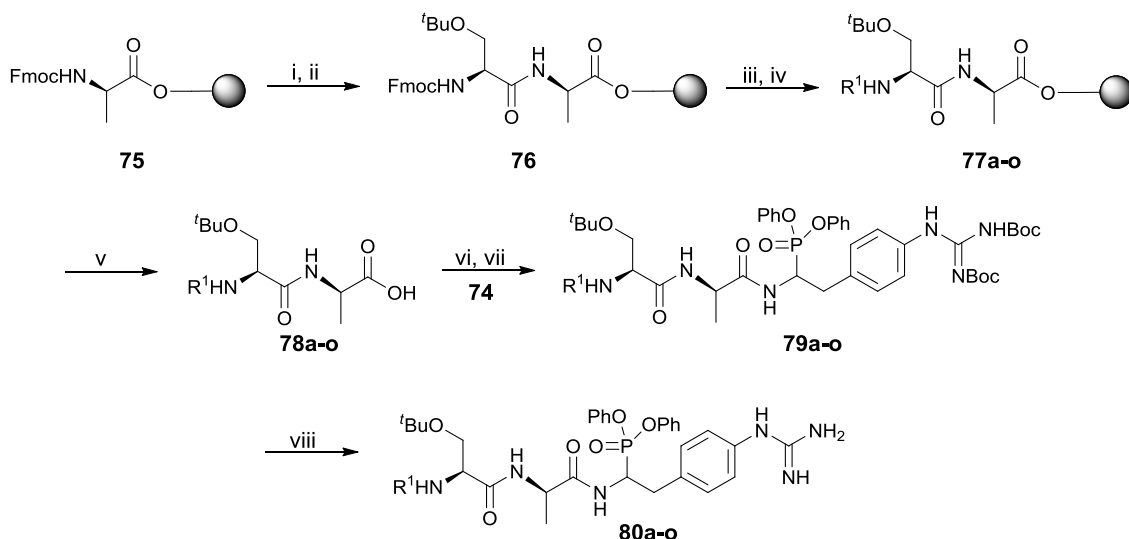
The synthesis of alafosfalin **69** (Scheme 14) begins with treating tributylstannylated α -amino alcohol with butyl lithium and diethyl chlorophosphate to give **65**. The alcohol of compound was deprotected to give **66**. Compound **66** underwent hydrogenolysis to give α -amino phosphonate **67**, which was coupled with Boc protected alanine using BOP as a coupling agent. The final step to form alafosfalin **69** involved deprotection of **68** using hydrogen bromide in acetic acid to remove the ester and amine protecting groups.

In other examples, Joossens *et al.*³¹ reported the synthesis of diphenyl phosphonate inhibitors against the urokinase plasminogen activator (uPA) using O-benzotriazole-1-yl-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) for peptide coupling. The group described a strategy for the synthesis of small libraries of tripeptidyl diphenylphosphonate analogues (Scheme 15) for structure-activity relationship studies.



Scheme 15 *Reagents and conditions:* (i) Boc_2O , Et_3N , dioxane, (ii) Dess-Martin; (iii) benzyl carbamate, triphenyl phosphite, $\text{Cu}(\text{OTf})_2$, CH_2Cl_2 ; (iv) TFA, (v) *N,N'*-bis(tert-butoxycarbonyl)-1-guanylpurazole, MeCN; (vi) H_2 , Pd/C.

The synthesis involves 2-(4-aminophenyl)ethanol **70** being Boc protected followed by oxidation of the alcohol to afford **71**. Aldehyde **71** is then involved in a three component reaction with benzyl carbamate and triphenyl phosphite to give **72**. Subsequent deprotection of **72** using trifluoroacetic acid (TFA) and reacting with *N,N'*-bis(tert-butoxycarbonyl)-1-guanylpurazole gave **73** that required deprotection using hydrogenolysis to give **74**.



Scheme 16 *Reagents and conditions:* (i) piperidine, DMF, (ii) Fmoc-(D)-Ser(O^tBu), TBTU, Et_3N , DMF; (iii) piperidine, DMF, (iv) $\text{R}^1\text{SO}_2\text{Cl}$, collidine, CH_2Cl_2 or R^1COOH , TBTU, Et_3N , DMF; (v) AcOH, MeOH, CH_2Cl_2 ; (vi) HOBt, resin-bound carbodiimide, CH_2Cl_2 , (vii) resin-bound polyamine; (viii) TFA.

With compound **74** prepared, the next step was to build up varied chains on a polymer support to couple with **74** to achieve final compounds **80** (Scheme 16). Fmoc protected resin bound alanine **75** was coupled with serine to give **76**. Deprotection of **76** followed by addition of various R¹ groups using sulfonyl chlorides or carboxylic acids afforded **77a-o**. Cleavage of **77a-o** from the resin gave **78a-o**, which was then coupled with **74** to give **79a-o**, followed by deprotection using TFA to give **80a-o**, with details of R¹ groups listed in Figure 4.

After synthesis, compounds **80a-o** were tested for inhibition of uPA and related enzymes (Table 9).

Joosens *et al.*³² also synthesised a series of irreversible diphenyl phosphonate inhibitors against urokinase plasminogen activator (uPA), which is important in vascular diseases and in cancer.

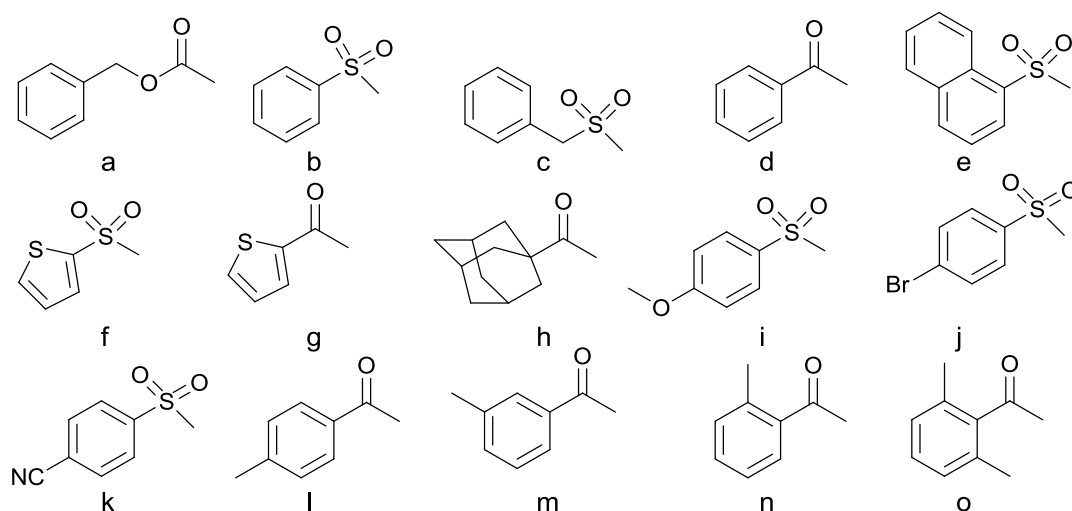
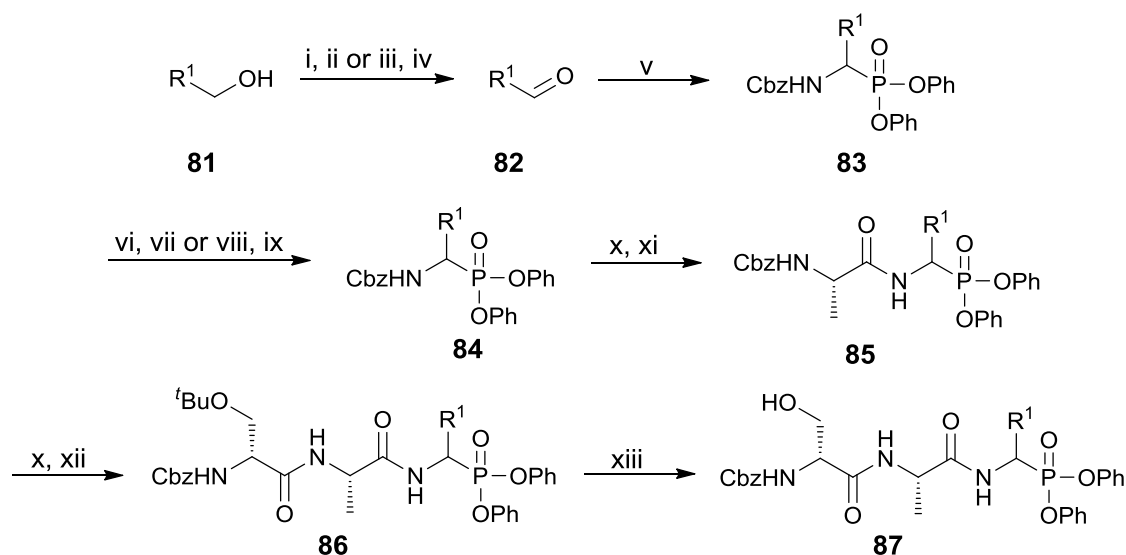


Figure 4 R¹ groups of compounds **80a-o**.

| No. | IC ₅₀ (nM) uPA | k _{app} (M ⁻¹ s ⁻¹) uPA | IC ₅₀ (μM)* | | | | |
|---|------------------------------|--|------------------------|-----------------------|----------------------|---------------------|-----------------------|
| | | | tPA | plasmin | thrombin | Factor Xa | trypsin |
| 80a | 4.3 ± 0.2 | 60 ± 2 x 10 ³ | 4.6 ± 0.7 (1000) | 2.0 ± 0.2 (470) | 20 ± 0.3 (4700) | 43% @ 125 | 0.3 ± 0.1 (70) |
| 80b | 2.6 ± 0.3 | 94 ± 4 x 10 ³ | 3.1 ± 0.7 (1200) | 1.9 ± 0.1 (740) | 4.9 ± 0.3 (1900) | 64% @ 250 | 0.16 ± 0.2 (60) |
| 80c | 3.5 ± 0.7 | 97 ± 7 x 10 ³ | 0.28 ± 0.02 (80) | 0.037 ± 0.003 (11) | 0.77 ± 0.03 (220) | 33 ± 5 (9400) | 0.31 ± 0.03 (90) |
| 80d | 5 ± 1 | 40 ± 2 x 10 ³ | 15 ± 2 (3000) | 15 ± 1 (3000) | 22 ± 1 (5400) | 45% @ 250 | 0.40 ± 0.05 (80) |
| 80e | 5.8 ± 1.2 | 46 ± 2 x 10 ³ | 4.4 ± 0.5 (760) | 0.99 ± 0.05 (155) | 6 ± 1 (1000) | 96 ± 14 (16551) | 0.280 ± 0.04 (50) |
| 80f | 1.5 ± 0.2 | 80 ± 10 x 10 ³ | 3.4 ± 0.1 (2300) | 1.54 ± 0.17 (1000) | 4.3 ± 0.2 (2800) | 68% @ 250 | 0.098 ± 0.006 (65) |
| 80g | 6.9 ± 0.8 | 71 ± 0.2 x 10 ³ | 13 ± 2 (1900) | 10 ± 1 (1400) | 35 ± 4 (5000) | ~250 | 280 ± 30 |
| 80h | 8 ± 1 | 36 ± 1 x 10 ³ | 8.6 ± 0.6 (1000) | 5 ± 1 (600) | 6 ± 0.1 (750) | 62% @ 250 | 0.16 ± 0.03 (20) |
| 80i | 5.8 ± 0.4 | 98 ± 6 x 10 ³ | 4.3 ± 0.2 (740) | 1.7 ± 0.3 (293) | 1.67 ± 0.04 (288) | 100 ± 18 (19000) | 0.36 ± 0.04 (60) |
| 80j | 6.6 ± 0.5 | 45 ± 1 x 10 ³ | 3.7 ± 0.2 (560) | 0.9 ± 0.1 (136) | 2.98 ± 0.03 (451) | 58 ± 7 (9000) | 0.24 ± 0.02 (36) |
| 80k | 6.2 ± 0.3 | 74 ± 4 x 10 ³ | 6.6 ± 0.5 (1100) | 0.9 ± 0.2 (145) | 7.5 ± 0.4 (1200) | 53% @ 250 | 0.44 ± 0.06 (70) |
| 80l | 7 ± 2 | 70 ± 10 x 10 ³ | 16 ± 1 (2300) | 5.0 ± 0.4 (700) | 67 ± 12 (9600) | 53% @ 250 | 0.16 ± 0.03 (23) |
| 80m | 4.4 ± 0.6 | 75 ± 10 x 10 ³ | 18 ± 3 (4000) | 8 ± 1 (1800) | 34 ± 4 (7700) | 56% @ 250 | 0.16 ± 0.03 (40) |
| 80n | 5 ± 1 | 83 ± 11 x 10 ³ | 5.5 ± 0.4 (1100) | 7.2 ± 0.5 (1400) | 15.9 ± 0.6 (3200) | 57% @ 250 | 0.14 ± 0.02 (30) |
| *IC ₅₀ (μM) or % inhibition at given concentration. Values in bracket are the selectivity index (IC ₅₀ / IC ₅₀ of uPA) | | | | | | | |

Table 9 Inhibition of uPA and related enzymes by phosphonate inhibitors

The synthesis of the inhibitors (**Scheme 17**) began with amine protection of various alcohols **81** followed by oxidation to give aldehydes **82**. Compounds **82** were then reacted with benzyl carbamate and triphenyl phosphite to afford **83**. The amine protecting groups in **83** were deprotected followed by coupling to protected guanylpurazole to give **84**. Compound **84** was deprotected using hydrogenolysis followed by coupling to protected alanine to give **85**. Compound **85** was deprotected and coupled to (D)-serine to give **86**. The final step required deprotection of the Boc groups to give **87**, with the details of R¹ groups used given in **Figure 5**.



R^1 groups are described in **Figure 5**

Scheme 17 Reagents and conditions: (i) Boc_2O , (ii) Dess-Martin; (iii) phthalic anhydride, Et_3N , toluene, 90°C , (iv) Swern oxidation; (v) benzyl carbamate, triphenyl phosphite, $\text{Cu}(\text{OTf})_2$, CH_2Cl_2 ; (vi) TFA, (vii) N,N' -bis(tert-butoxycarbonyl)-1-guanylpurazole, MeCN; (viii) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$, THF, (ix) N,N' -bis(tert-butoxycarbonyl)-1-guanylpurazole, MeCN; (x) H_2/Pd , (xi) Cbz-Ala-OH, TBTU, DMF; (xii) Cbz-(D)-Ser(O^tBu), TBTU, DMF; (xiii) TFA.

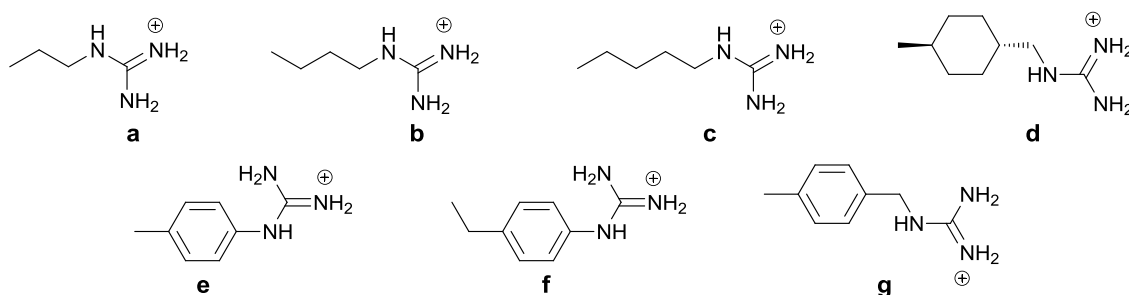
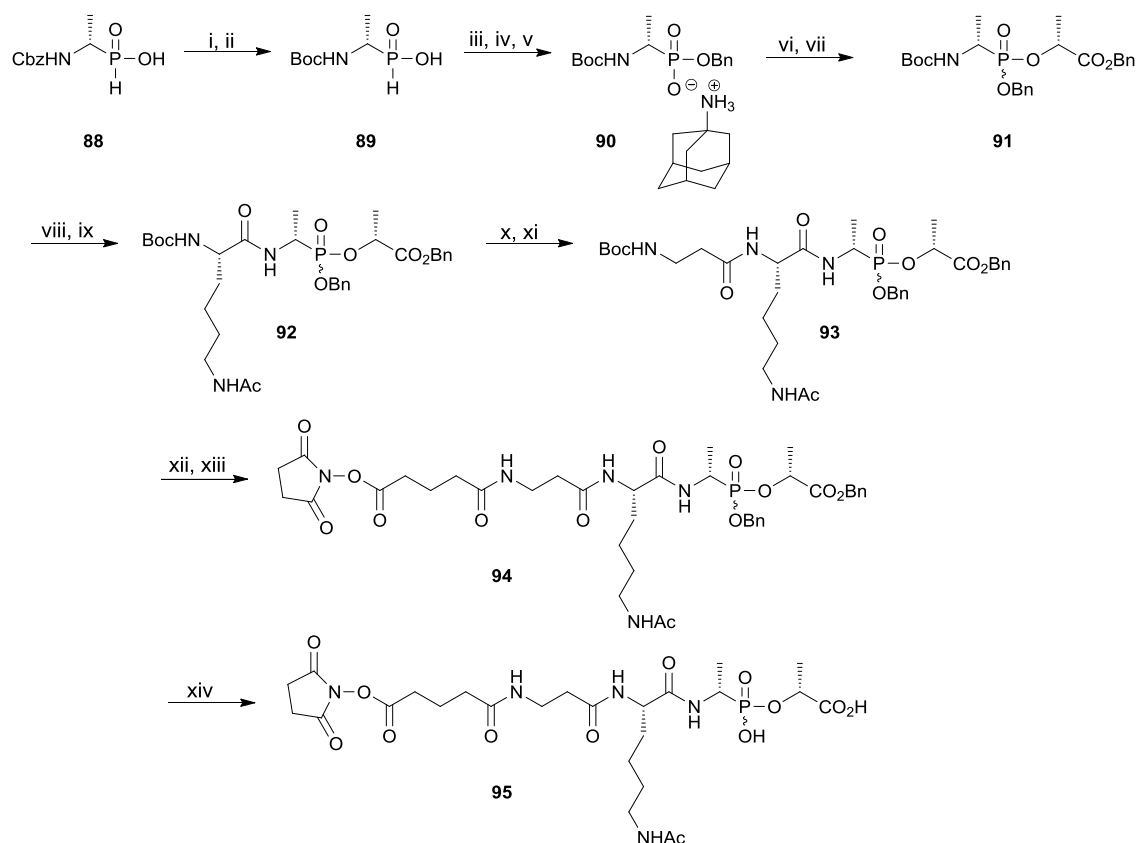


Figure 5 R^1 groups for compounds **87a-g**.

Isomura *et al.*³³ synthesised phosphinopeptides to help in their work related to the immune response associated with the problems of vancomycin resistance. The work involved synthesising a phosphonate peptidomimetic as outlined in the following scheme (**Scheme 18**).



Scheme 18 *Reagents and conditions:* (i) 30% HBr; (ii) Boc₂O; (iii) DCC, BnOH; (iv) NaIO₄ (v) 1-adamantine; (vi) aq. HCl; (vii) benzyl D-lactate, BOP, DIPEA; (viii) TFA, CH₂Cl₂; (ix) Boc-Lys(Ac)-OH, HBTU, NMM; (x) TFA, CH₂Cl₂; (xi) Boc-β-Ala-OH, HBTU, NMM; (xii) TFA, CH₂Cl₂; (xiii) *N*-hydroxysuccinimidylhemiglutaryl chloride, DIPEA, CH₂Cl₂; (xiv) H₂, Pd/C, MeOH.

The synthesis begins with the deprotection and protection of the amine in **88** using 30% hydrobromic acid to remove Cbz followed by Boc protection of the amine to give **89**. Subsequent benzylation of the alcohol of **89** followed by treatment with sodium periodate then addition of 1-adamantamine gave the corresponding salt **90**. Reacting **90** with benzyl (D)-lactate gave **91**. The Boc group in **91** was deprotected with TFA followed by coupling with Boc protected lysine with an acetyl on the terminal amine chain to give **92**. Further deprotection of the Boc in **92** was carried out followed by coupling to β-alanine to give **93**. Deprotection of the Boc group in **93** followed by coupling to *N*-hydroxysuccinimidylhemiglutaryl chloride gave **94**. Compound **94** was treated under hydrogenolysis to give the free phosphinic and carboxylic acids **95**.

Compound **95** was subsequently tested against vancomycin resistant strain VCA33H3 (**Table 10**) but showed no activity.

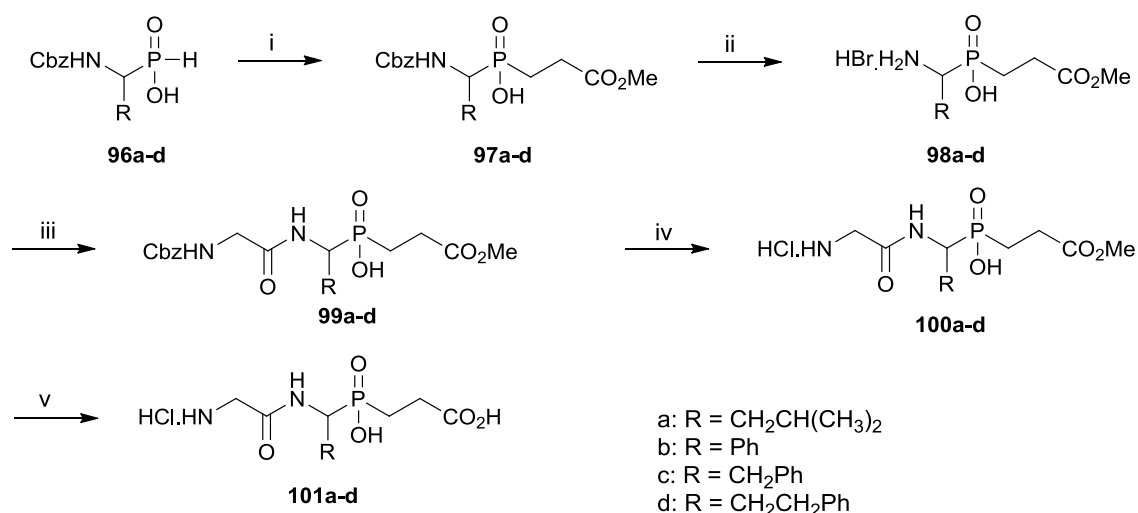
| K_{cat} (h^{-1}) | K_{m} (mM) | $k_{\text{cat}} / K_{\text{m}}$ ($\text{M}^{-1} \text{min}^{-1}$) | $k_{\text{cat}}/k_{\text{uncat}}$ | K_{i} (μM) |
|---|------------------------|--|-----------------------------------|-------------------------------------|
| 0.120 | 2.17 | 0.921 | 530 | 160 |

Table 10 Kinetic data for VCA33H3 that cleaves (D)-Ala-(D)-Lac bond in model peptidoglycan substrate.

1.3. Reaction involving Michael addition to acrylates

The most straight forward and common route to phosphonopeptide in which the amide linkage is replaced by a $\text{P}(\text{O})\text{-CH}_2$ moiety is by Michael addition of a phosphinic/phosphinous acid to appropriate acrylates.

Mucha *et al.*³⁴ used this method in the syntheses of a number of phosphinic tripeptide analogues and evaluated their biological activity against cathepsin C (dipeptidyl peptidase I), a papain-like cysteine aminopeptidase that is expressed in the lysosomes of several tissues and plays an important role in tumorigenesis (**Scheme 19**).



Scheme 19 Reagents and conditions: (i) HMDS, 100-110 °C, 3 h then $\text{H}_2\text{C}=\text{CHCO}_2\text{Me}$; (ii) 33% HBr/AcOH, rt, 2 h; (iii) CbzNHCH₂CO₂H, isobutyl chloroformate, Et₃N, 0 °C to rt; (iv) 33% HBr/AcOH, rt, 2 h; evaporation, 10% HCl and washing with ether; (v) TMSBr 10 eq., 3 d, rt, then H₂O, then conc. HCl.

The synthesis of the phosphorus tripeptides begins with the formation of the phosphinic amino acid analogue **96**. Compound **96** was treated with HMDS followed by addition of methyl acrylate to give the conjugate addition product **97**. Deprotection of the Cbz group using hydrogen bromide in acetic acid gave **98**, which was then coupled with the mixed anhydride of Cbz protected glycine, which had been generated *in situ* by treatment of the acid with isobutyl chloroformate, to give **99**. Deprotection of **99** using hydrogen bromide and acetic acid followed by treatment with 10% hydrochloric acid gave **100**. The methyl ester in **100** was hydrolysed with bromotrimethylsilane (TMSBr) to give **101**.

| No. | K _i [mM] | No. | K _i [mM] |
|-------------|---------------------|-------------|---------------------|
| 100a | 0.040 | 101a | 0.039 |
| 100b | 0.188 | 101b | 0.176 |
| 100c | 0.514 | 101c | 0.429 |
| 100d | 0.312 | 101d | 0.187 |

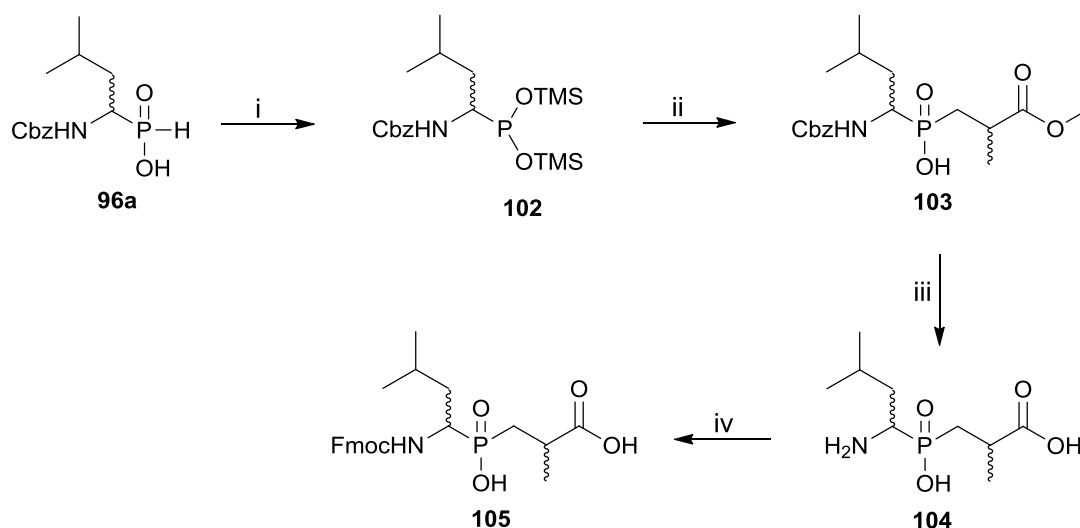
Table 11 Inhibition of cathepsin C by phosphinic tripeptide analogues.³⁴

The synthesised compounds were tested for their activity towards inhibition of cathepsin C (**Table 11**). They were shown to be reversible, non-competitive, slow-binding inhibitors.

Manzenrieder *et al.*³⁵ have reported their work on the synthesis of phosphino analogues of OM-003 an inhibitor of human β -secretase (BACE1). BACE1 is an aspartic protease that initiates the processing of amyloid precursor protein (APP) which generates amyloid fibrils that are deposited in the cerebrum, a major factor in the pathogenesis of Alzheimer's disease.

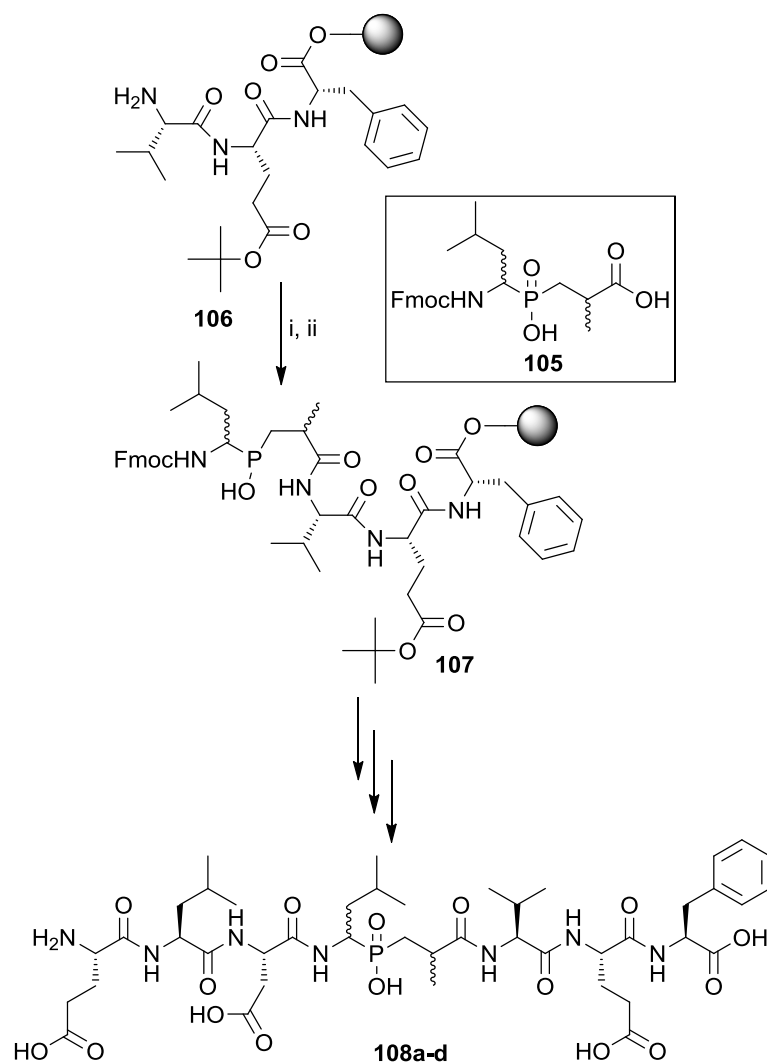
The synthesis of the inhibitors (**Scheme 20**) begins with activation of amino phosphinate **96a** using HMDS to form a bis(trimethylsilyl) phosphonite **102** followed by treatment with methyl methacrylate to give **103**. Treating **103** with hydroiodic acid deprotects the Cbz and methyl ester to give **104**. The final step of the synthesis involved

protection of the amine with FmocCl to give the desired compound **105** as a mixture of diastereomers.



Scheme 20 *Reagents and conditions:* (i) 5 eq. HMDS, 100-110 °C, 2 h (Ar); (ii) 60 °C, 1.25 eq. methyl methacrylate, 85-90 °C, 3h, (Ar) 91%; (iii) 57% aq HI, 2 h; (iv) 40% Na₂CO₃ (dioxane), 0 °C, 1.2 eq. FmocCl, 12 h (74% 2 steps).

Once **105** had been synthesised it was used to make compounds **108a-c** (**Scheme 21**). First, a three amino acid sequence H₂N-Val-Glu(O-^tBu)-Phe-OH was synthesised on a resin to give **106**. Coupling of polymer supported amino acid **106** with **105** was carried out using (pentafluorophenyl) oxytripyrrolidinophosphonium hexafluorophosphate (PyPOP), diisopropylethylamine (DIPEA), *N*-methylpyrrolidine (NMP) to give **107**. After formation of **107**, aspartate, leucine and glutamate were sequentially coupled followed by cleavage from the resin followed by separation by HPLC to give three fractions of the four stereoisomers **108a-d**.



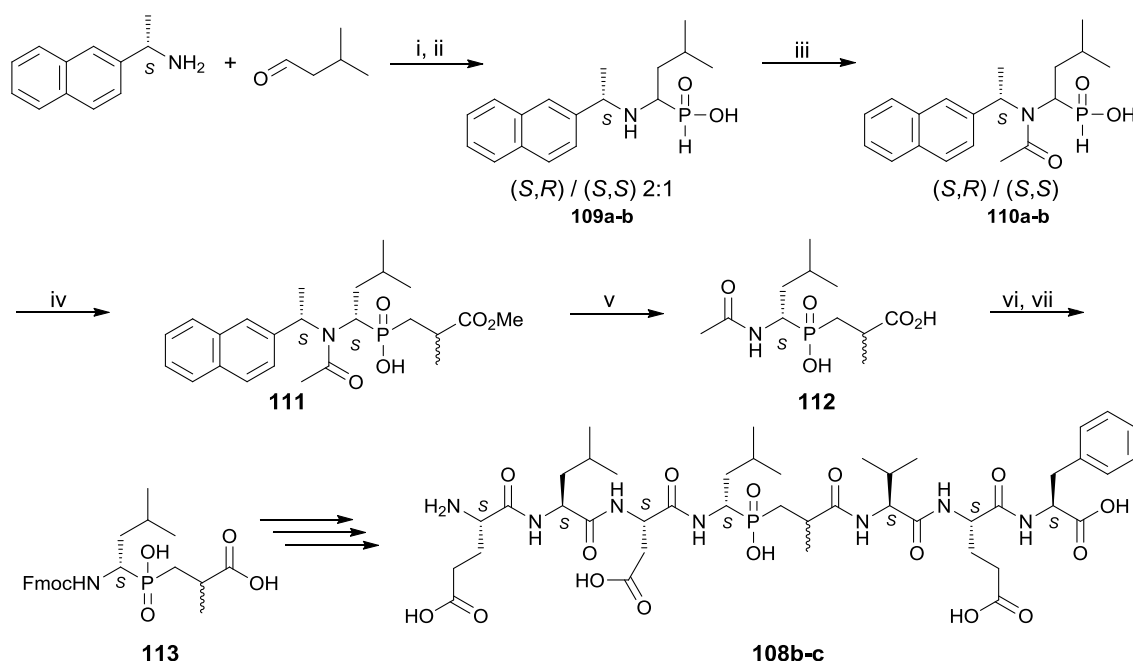
Scheme 21 Reagents and conditions: (i) 2.5 eq. **105**, 2.5 eq. PyPOP, 5 eq. DIPEA, NMP; (ii) wash with NMP.

| No. | Inhibition IC ₅₀ (nM) | Retention time (min) | No. of diastereomers |
|---------------|-------------------------------------|-------------------------|-------------------------|
| OM00-3 | 6 (±0.7) | - | 1 |
| 108a | 12 (±2) | 11.4 | 1 |
| 108b-c | 675 (±197) | 12.6, 13.1 | 2 |
| 108d | 2020 (±673) | 13.7 | 1 |

Table 12 IC₅₀ values (nM) of the isolated diastereomers against BACE1.

The phosphino peptides were tested for inhibition against BACE1 using OM00-3 (Glu-Leu-Asp-Leu-Ala-Val-Glu-Phe) as a standard (**Table 12**). It can be seen from the results that only one of the four diastereomers, **108a**, was potent.

The group had assumed the configuration of the non-fixed stereocentres in **108a** were (*R*) and (*S*), corresponding to (*S*) and (*S*) configuration in the native peptide. In order to confirm the stereochemistry a method was used to give fixed stereochemistry at the isobutyl group (**Scheme 22**). (*S*)-1-(2-naphthyl)-ethylamine was condensed with isovaleraldehyde to give the imine that was added to hypophosphorus acid to give phosphinic acids **109a-b** (2:1/*SR:SS*). **109a-b** were then acetylated using acetyl bromide (AcBr) to afford **110a-b**. Activation of **110b** using HMDS followed by reaction with methyl methacrylate to give **111**. Treatment of **111** with hydroiodic acid removed the chiral auxiliary to give **112**. Deprotection of the methyl ester and acetyl group followed by N-protection with FmocCl gave **113** an analogue of **105** with fixed stereochemistry. Further peptide synthesis was carried out as previously (**Scheme 21**). After cleavage from the solid support, compounds **108a-c** were obtained and identified by high-performance liquid chromatography (HPLC) retention time confirming that the absolute configuration of the active stereoisomer **108a** is indeed (*S*) at the stereocenter adjacent to the phosphorus atom.



Scheme 22 *Reagents and conditions:* (i) MgSO_4 , 0 °C, 1 h (Ar), C_6H_6 ; (ii) 5 eq. H_3PO_2 , 0 °C, overnight (Ar), (THF), (40% 2steps); (iii) 3 eq. Et_3N , 0 °C, 2 h (Ar), (THF), 1.5 eq. AcBr , rt, overnight (Ar), (THF) (73-94%); (iv) 5 eq. HMDS , 100-110 °C, 2 h (Ar), 60 °C, 1.25 eq. methyl methacrylate, 85-90 °C, 3 h (Ar) (85%); (v) 57% aq HI , 100 °C, 2 h (62%); (vi) 8N HCl , 100 °C, 12 h; (vii) sat. Na_2CO_3 , dioxane, 0 °C, 1.2 eq. FmocCl , 12 h (56% 2 steps).

Further testing of **108a** against other aspartic proteases was carried out (**Table 13**), however no selectivity was found against other proteases.

| No. | BACE1 | BACE2 | cathepsin D | Pepsin |
|-------------|----------------|----------------|----------------|---------------|
| 108a | 12 (± 2) | 12 (± 1) | 28 (± 8) | 8 (± 4) |

Table 13 IC_{50} values (nM) of **108a** against BACE1, BACE2, cathepsin D and Pepsin.

Vassiliou and co-workers have also synthesised a number of phosphinic pseudo-tripeptides for use as inhibitors of MMPs, which are important in extracellular matrix remodelling.³⁶

The work focuses on two O-adamantyl phosphinic esters (**Figure 6**) that can be synthesised from precursor phosphinic acid **115** (**Scheme 23**) and used in further peptide chain extensions.

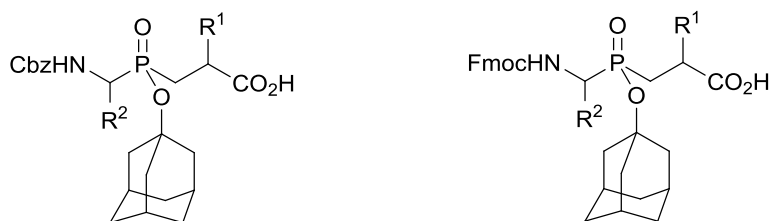
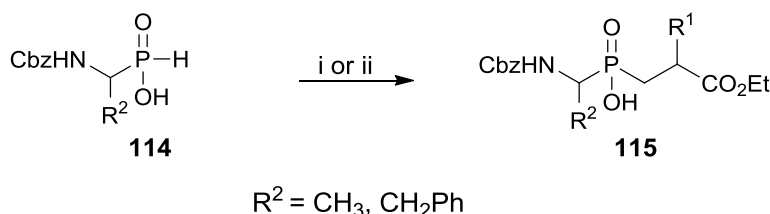


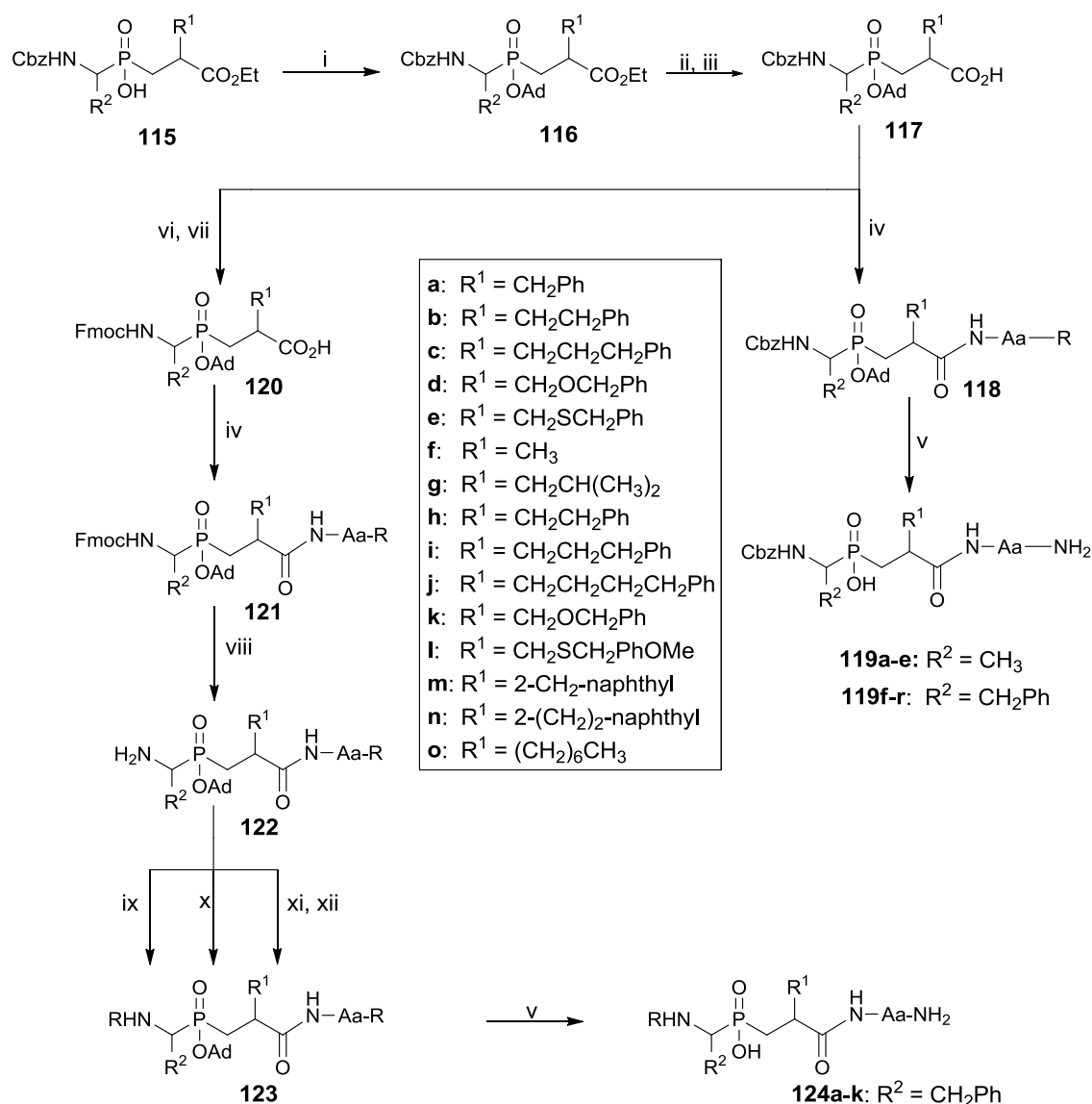
Figure 6 O-adamantyl phosphinic esters



Scheme 23 Reagents and conditions: (i) HMDS, 110 °C, $\text{H}_2\text{C}=\text{C}(\text{R}^3)\text{CO}_2\text{C}_2\text{H}_5$, EtOH, 70 °C; (ii) TMSCl, 0 °C, rt, $\text{H}_2\text{C}=\text{C}(\text{R}^3)\text{CO}_2\text{C}_2\text{H}_5$, rt, EtOH.

Key intermediates **115**, were prepared by a Michael addition of R^1 -substituted acrylates with mono substituted phosphinates **114**. Compounds **115** were then used to synthesise a series of phosphinic pseudo-tripeptides (**Scheme 24**).

Precursor **115** is reacted with silver oxide forming the silver salt, followed by treatment with adamantyl bromide to give **116**. The methyl ester of **116** was hydrolysed using a mixture of sodium hydroxide in MeOH then neutralised with hydrochloric acid to give compound **117** selectively without removal of the adamantyl group. Chain extension of **117** with the amino function of an amino acid (aa) chain, preloaded onto a Rink amide resin, using *O*-benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate (HBTU), DIPEA, NMP formed compound **118**. Of course, compound **118** could be further used in solid-phase syntheses, but in this case, concurrent hydrolysis of the adamantyl ester and release from the resin using TFA/ CH_2Cl_2 /triisopropylsilane/water gave the corresponding phosphinic tripeptides **119a-r** with varied R^2 and R^1 groups as shown (**Scheme 24**).



Scheme 24 *Reagents and conditions:* (i) AdBr, Ag₂O, CHCl₃; (ii) 4N NaOH, MeOH; (iii) aq. HCl; (iv) H₂N-Aa-Rink (R), HBTU, DIPEA, NMP; (v) TFA/CH₂Cl₂/triisopropylsilane/H₂O 5:4:0.5:0.5; (vi) HCOO⁻ NH₄⁺, 10% Pd/C, MeOH; (vii) FmocCl, 20% Na₂CO₃, dioxane; (viii) 20% piperidine/NMP; (ix) RCO₂H, HBTU, DIPEA, NMP; (x) RCOCl, DIPEA, CH₂Cl₂; (xi) BrCH₂COBr, DIPEA, CH₂Cl₂; (xii) aniline derivatives, DIPEA, DMSO.

To facilitate chain extension on both the C- and N-terminus the first step is to remove the Cbz protecting group by reduction using Pd/C in methanol with ammonium formate as a source of hydrogen. This is then treated with FmocCl in dioxane using sodium carbonate to introduce the Fmoc protecting group to give compound **120** that is more suitable for peptide synthesis. Reacting **120** with a Rink amide resin as previously discussed allows for the chain extension at the C-terminus giving compound **121**. Deprotection of the Fmoc group of **121** using piperidine/NMP gives amine **122**. The

amine can be reacted under three different conditions as shown to give varied phosphinic pseudopeptides **123**. The last step of the synthesis is the concurrent cleavage from the Rink resin and removal of the adamantyl protecting group as previously mentioned to give **124a-k**.

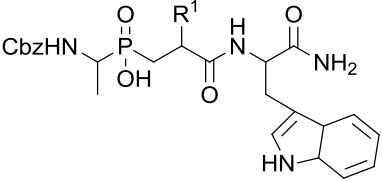
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|--|--|----------------|----------------|----------------|-------------------|--------------|---------------------|--------------|
| K_i (nM)* | | | | | | | | |
| No. | R ¹ | MMP-11 mST3 | MMP-2 gel-A | MMP-9 gel-B | MMP-14 MT1-MMP | MMP-1 HFC | MMP-7 matrilysin | MMP-8 HNC |
| 119a | CH ₂ Ph | 350 | 250 | 280 | 2030 | 24% @ 2μM | 8% @ 2μM | 240 |
| 119b | CH ₂ CH ₂ Ph | 51 | 80 | 60 | 270 | 23% @ 2μM | 3% @ 2μM | 20 |
| 119c | CH ₂ CH ₂ CH ₂ Ph | 100 | 31 | 23 | 92 | 30% @ 2μM | 4% @ 2μM | 8 |
| 119d | CH ₂ OCH ₂ Ph | 175 | 250 | 44 | 550 | 15% @ 2μM | 1% @ 2μM | 19 |
| 119e | CH ₂ SCH ₂ Ph | 36 | 14 | 6 | 26 | 45% @ 2μM | 2% @ 2μM | <0.5 |
| 119f | CH ₃ | 2670 | 0% @ 2μM | 0% @ 2μM | 0% @ 2μM | 20% @ 2μM | 0% @ 2μM | 9% @ 2μM |
| 119g | CH ₂ CHMe ₂ | 22 | 202 | 65 | 192 | 45% @ 2μM | 210 | 40 |
| 119h | CH ₂ CH ₂ Ph | 8.8 | 275 | 110 | 660 | 10% @ 2μM | 7% @ 2μM | 45 |
| 119i | CH ₂ (CH ₂) ₂ Ph | 5 | 20 | 10 | 105 | 23% @ 2μM | 8% @ 2μM | 2.5 |
| 119j | CH ₂ (CH ₂) ₃ Ph | 33 | 145 | 70 | 580 | 5% @ 2μM | 7% @ 2μM | 4.3 |
| 119k | CH ₂ OCH ₂ Ph | 16 | 85 | 55 | 545 | 9% @ 2μM | 3% @ 2μM | 20 |
| 119l | CH ₂ -S-CH ₂ -Ph-OMe | 2 | 6 | 3 | 22 | 13% @ 2μM | 20% @ 2μM | 0.7 |
| 119m | 2-CH ₂ -naphthyl | 74 | 330 | 675 | 1350 | 0% @ 2μM | 1800 | 230 |
| 119n | 2-(CH ₂) ₂ -naphthyl | 12 | 30 | 55 | 125 | 0% @ 2μM | 2100 | 34 |
| 119o | (CH ₂) ₆ -CH ₃ | 34 | 75 | 30 | 271 | 27% @ 2μM | 4% @ 2μM | 6 |

Table 14 Influence of the R¹ Substituent (P₁' Position) on MMP inhibition (*K_i (nM) or % inhibition at given concentration).

After their synthesis the compounds were tested for activity against MMPs (**Table 14**, **Table 15**, and **Table 16**). Variations in the substituents used in the R¹ position were carried out to see the effect on potency (**Table 14**). When the R¹ substituent is an unusual amino acid side chain the compounds are potent inhibitors against five MMPs, with the introduction of sulphur into the side chain giving a large increase in potency. Use of the naphthyl group increased potency showing the S₁' pocket is large enough to accept a bulky group.

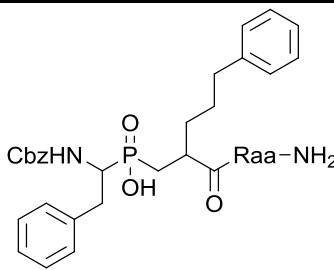
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|--|-------------|----------------|----------------|----------------|-----------------------|--------------|---------------------|--------------|
| K _i (nM)* | | | | | | | | |
| No. | Raa | MMP-11 mST3 | MMP-2 gel-A | MMP-9 gel-B | MMP-14 MT1- MMP | MMP-1 HFC | MMP-7 matrilysin | MMP-8 HNC |
| 119p | Ala | 20% @ 1μM | 31% @ 2μM | 45% @ 2μM | 2960 | 9% @ 2μM | 0% @ 2μM | 240 |
| 119i | (L)- Trp | 5 | 20 | 10 | 105 | 23% @ 2μM | 8% @ 2μM | 2.5 |
| 119q | (D)- Trp | 1% @ 1μM | 6% @ 2μM | 14% @ 2μM | 10% @ 10μM | 0% @ 2μM | 0% @ 2μM | 2% @ 1μM |
| 119r | Dpa | 27 | 260 | 245 | 1282 | 9% @ 2μM | 4% @ 2μM | 25 |

Table 15 Influence of the Raa Residue (P₂' Position) on MMP inhibition (*K_i (nM) or % inhibition at given concentration).

Modifications to P₂' were carried using of natural and unnatural tryptophan, alanine and an aromatic side chain with activities shown (**Table 15**). Variations in the P₂ position gave varied results as shown (**Table 16**).

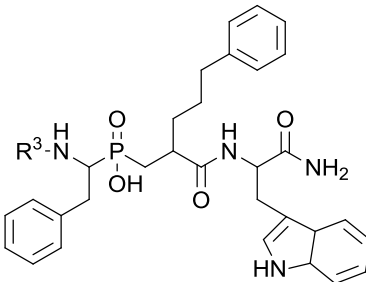

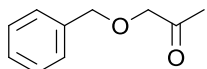
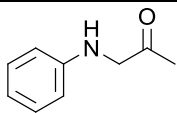
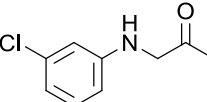
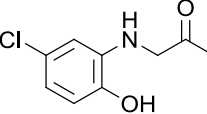
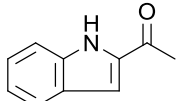
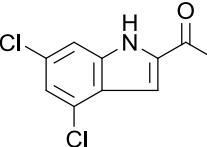
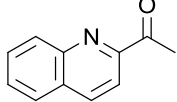
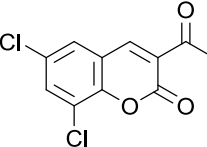
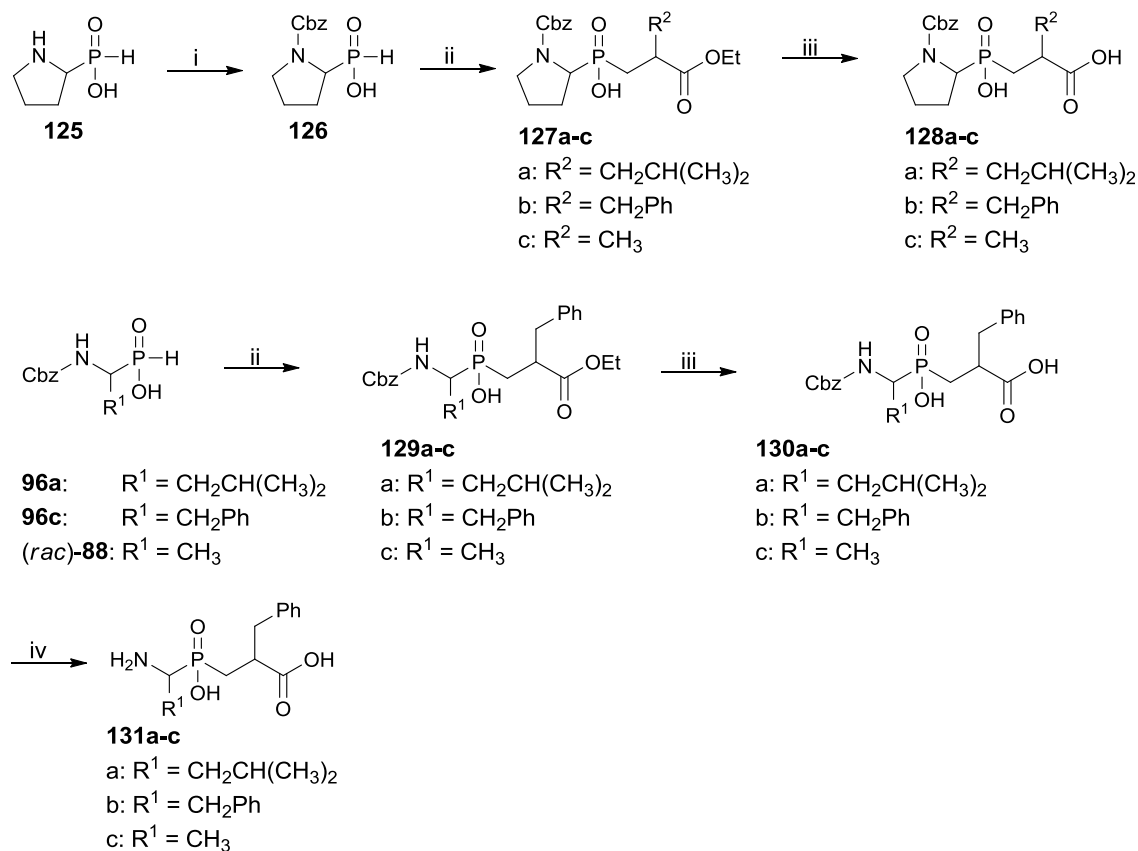
|  | | | | | | | | |
|--|---|----------------|----------------|----------------|-------------------|--------------|---------------------|--------------|
| K_i (nM)* | | | | | | | | |
| No. | R^3 | MMP-11 mST3 | MMP-2 gel-A | MMP-9 gel-B | MMP-14 MT1-MMP | MMP-1 HFC | MMP-7 matrilysin | MMP-8 HNC |
| 124a |  | 20 | 26 | 35 | 90 | 43% @ 2μM | 28% @ 2μM | 3.5 |
| 124b |  | 5 | 8 | 10 | 40 | 43% @ 2μM | 52% @ 2μM | 2.5 |
| 124c |  | 15 | 17 | 6 | 73 | 49% @ 2μM | 28% @ 2μM | 4.5 |
| 124d |  | 3.8 | 9 | 6 | 45 | 77% @ 2μM | 40% @ 2μM | 4 |
| 124e |  | 10 | 30 | 34 | 63 | 72% @ 2μM | 65% @ 2μM | 7.5 |
| 124f |  | 1.5 | 10 | 8 | 41 | 53 | 605 | 1.5 |
| 124g |  | 0.9 | 24 | 7 | 32 | 36 | 117 | 5 |
| 124h |  | 4.2 | 19 | 13 | 60 | 340 | 370 | 5 |
| 124i |  | 5 | 100 | 110 | 217 | 62% @ 2μM | 52% @ 2μM | 17 |
| 124j | Cbz-Ala | 8 | 11 | 10 | 41 | 40% @ 2μM | 33% @ 2μM | 5.5 |
| 124k | Cbz-Leu | 6 | 40 | 22 | 53 | 32% @ 2μM | 45% @ 2μM | 7 |

Table 16 Influence of the R^3 Substituent (P_2 Position) on MMP inhibition (* K_i (nM) or % inhibition at given concentration).

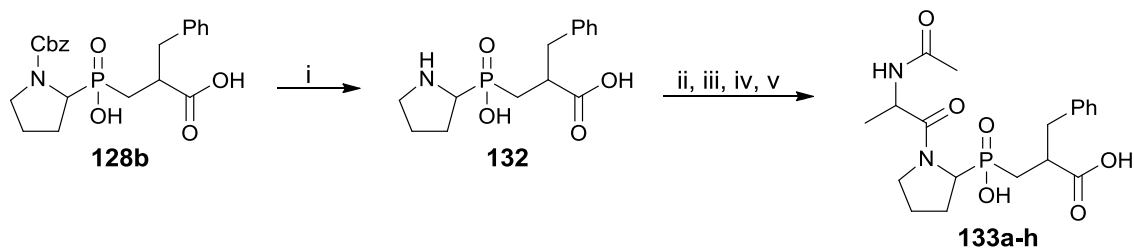
Mores *et al.*³⁷ developed a number of phosphinic peptide inhibitors of angiotensin-converting enzyme 2 (ACE2) and tested them for inhibition.



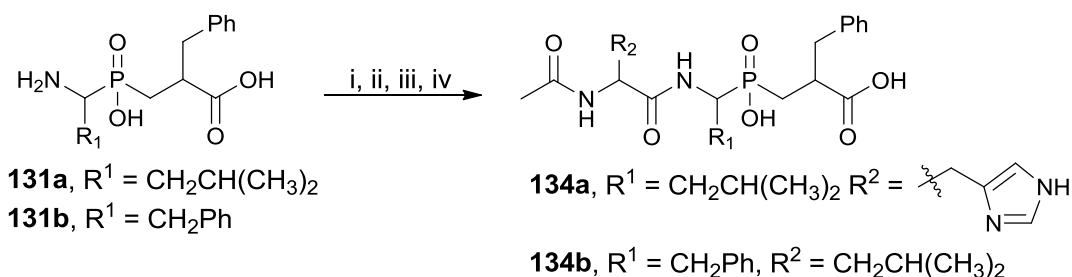
Scheme 25 Reagents and conditions: (i) aq. Na_2CO_3 , CbzCl 1.8 eq., 0 °C to rt, 12 h, 80%; (ii) HMDS 5 eq., 110 °C, 1 h, Ar, $CH_2=C(R^2)CO_2Et$ 1.2 eq., 4 h, then EtOH; **138a-c** 52%, 47%, 49%, **141a-c** 72%, 81%, 74% (iii) 1M NaOH in MeOH, rt, 12 h; **139a-c** 95%, 96%, 92%, **142a-c** 88%, 90%, 95% (iv) H_2 , 10% Pd/C, MeOH/ H_2O 4:1, rt, 2 h; **143a-c** 92%, 95%, 94%.

The phosphinic inhibitors are built up through two possible routes (**Scheme 25**). Phosphinic acid **125** is protected to give **126**. Compound **126** is activated using HMDS then reacted with various acrylates to give **127a-c**. The ester in **127a-c** is hydrolysed to give compounds **128a-c**. Phosphinic acids **96a**, **96c** and **88** are also reacted with acrylates to give **129a-c**. The esters in **129a-c** are hydrolysed as per **128a-c** to give **130a-c**. The final step involves deprotection of **130a-c** to yield **131a-c**.

Compound **128b** (**Scheme 26**) was deprotected by hydrogenolysis to give **132**. Compound **132** was then coupled with various protected amino acids followed by deprotections and acetylation to give **133a-h**.



Scheme 26 *Reagents and conditions:* (i) H_2 , 10% Pd/C, MeOH/ H_2O 4:1, rt, 2 h, 95%; (ii) RNH-aa- CO_2Np 1.5 eq., Et_3N 2 eq. in DMF, rt, 12 h, 70-80%; (iii) TFA/ CH_2Cl_2 , 50%, rt, 1 h, 90-95% or H_2 , 10% Pd/C, MeOH/ H_2O 4:1, rt, 2 h, 90-95% or $\text{HCO}_2\text{H}/\text{CH}_2\text{Cl}_2$, 5%, rt, 10 min, 95%; (iv) Ac_2O 3 eq. in py, rt, 12 h, 70-75%; (v) TFA/ CH_2Cl_2 , 50%, rt, 1 h, 90-95%; aa = a: alanine, b: leucine, c: lysine, d: glutamic acid, e: tyrosine, f: phenylalanine, g: valine, h: histidine; (Np = *p*-nitrophenyl).

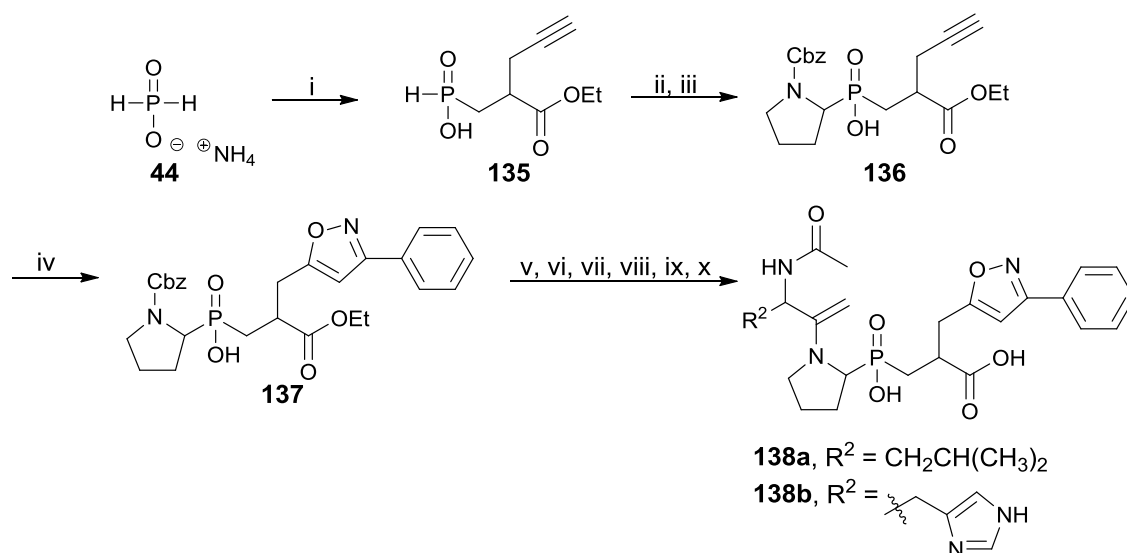


Scheme 27 *Reagents and conditions:* (i) BocLeuONp, Et_3N , DMF, rt, 12 h / TrtHis(Trt)ONp; (ii) TFA/ CH_2Cl_2 50%, rt, 1 h / $\text{HCO}_2\text{H}/\text{CH}_2\text{Cl}_2$ 5% 10 min; (iii) Ac_2O , Pyridine, rt, 12 h; (iv) TFA/ CH_2Cl_2 , 50% 1 h; (Np = *p*-nitrophenyl).

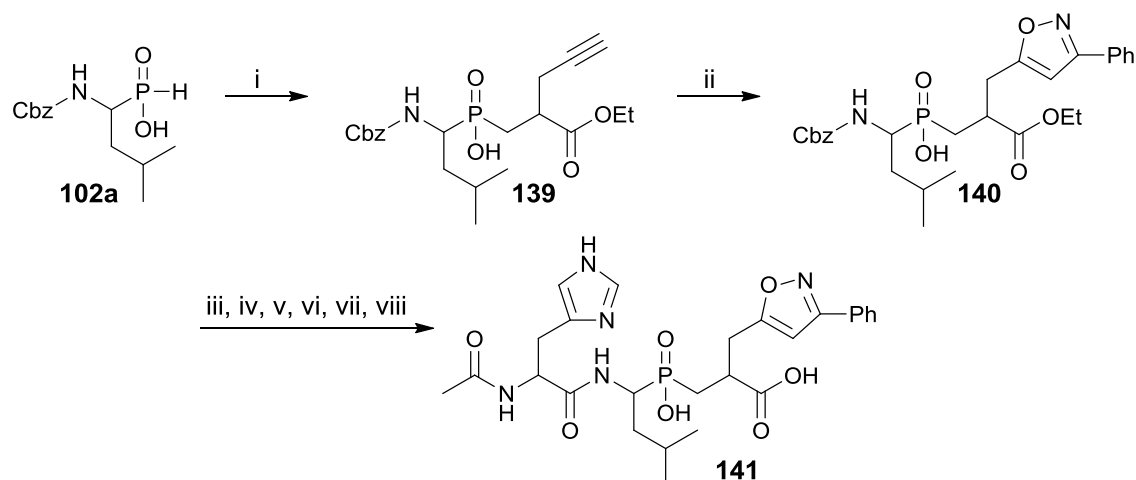
Compounds **131a-b** (Scheme 27) were coupled with protected amino acids followed by deprotection and acetylation to give **134a-b**.

The synthesis of compounds similar to **133a-h** was carried out with inclusion of an alkyne in order to be able to form oxazole compounds **138a-b** (Scheme 28).

Ammonium phosphinate **44** was activated using HMDS then reacted with an alkyne containing acrylate to afford **135**. Compound **135** was then reacted with 3,4-dihydro-2*H*-pyrrole which was subsequently protected to give **136**. Compound **136** underwent a cycloaddition to give **137**. The ester in **137** was hydrolysed, then the pyrrole was deprotected and the free amine coupled to a protected amino acid followed by N-acetylation and finally deprotection to give **138a-b**.



Scheme 28 *Reagents and conditions:* (i) HMDS 1 eq., 110 °C, 1 h, Ar, $\text{CH}_2=\text{C}(\text{CH}_2\text{C}\equiv\text{CH})\text{CO}_2\text{Et}$ 0.2 eq. in CH_2Cl_2 , 0 °C to rt, 12 h, then EtOH, 88%; (ii) TMSCl 4 eq., Et_3N , 4 eq. in CH_2Cl_2 , 0 °C to rt, 3 h (Ar), 3,4-dihydro-2*H*-pyrrole 1.1 eq., 0 °C to rt, 1 h, then EtOH; (iii) MgO 3 eq., CbzCl 1.5 eq. in $\text{H}_2\text{O}/\text{Et}_2\text{O}$, 0 °C to rt, 12 h, 54% for two steps; (iv) $\text{RCH}=\text{NOH}$ 3 eq., py 0.74 eq. in CHCl_3 , 45 °C, 3 h, then **136**, Et_3N 3 eq., 45 °C, 3 d, repeat 3 times, 80%; (v) 1M NaOH in MeOH, rt, 12 h, 90%; (vi) H_2 , 10% Pd/C in MeOH/ H_2O 4:1, rt, 2 h, 90%; (vii) TrtHis(Trt)ONp 72% or BocLeuONp 75% 1.5 eq., Et_3N 2 eq. in DMF, rt, 12 h; (viii) TFA/ CH_2Cl_2 , 50%, rt, 1 h, 90% or $\text{HCO}_2\text{H}/\text{CH}_2\text{Cl}_2$ 5%, rt, min, 92%; (ix) Ac_2O 3 eq. in py, rt, 12 h, 70-75%; (x) TFA/ CH_2Cl_2 , 50%, rt, 1 h, 90% for **138b**; (Np = *p*-nitrophenyl).



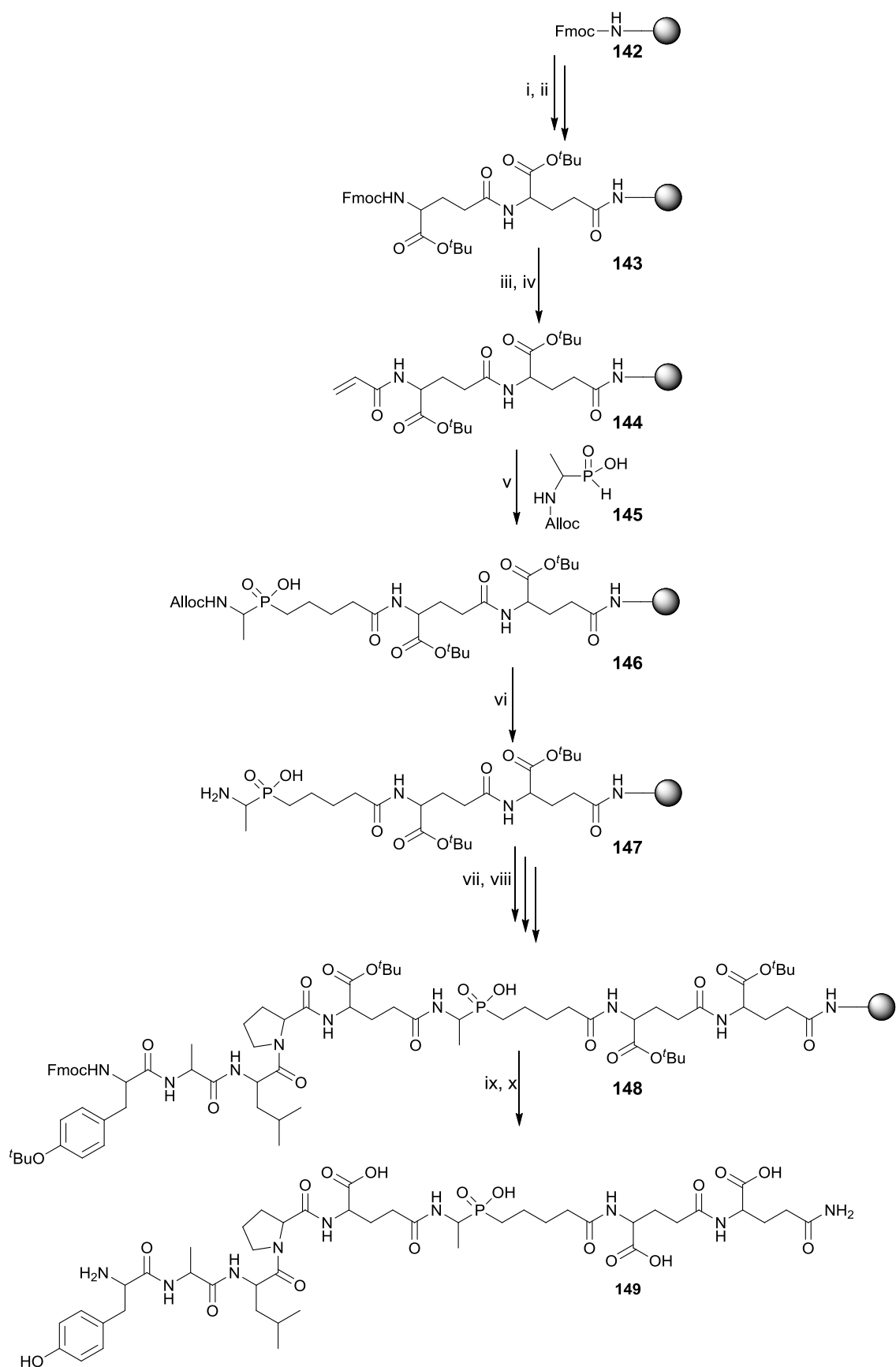
Scheme 29 *Reagents and conditions:* (i) HMDS 1 eq., 110 °C, 1 h (Ar), $\text{CH}_2=\text{C}(\text{CH}_2\text{C}\equiv\text{CH})\text{CO}_2\text{Et}$ 1.2 eq., 4 h, then EtOH, 98%; (ii) $\text{PhCH}=\text{NOH}$ 3 eq., NCS 3 eq., py 0.74 eq. in CHCl_3 , 45 °C, 3 h, then **151**, Et_3N 3 eq., 45 °C, 3 d, repeat 3 times, 83%; (iii) 1M NaOH in MeOH, rt, 12 h, 92%; (iv) H_2 , 10% Pd/C in MeOH/ H_2O 4:1, rt, 2 h, 95%; (v) TrtHis(Trt)ONp 1.5 eq., Et_3N 2 eq. in DMF, rt, 12 h, 78%; (vi) $\text{HCO}_2\text{H}/\text{CH}_2\text{Cl}_2$ 5%, rt, 1 h, 93%; (vii) Ac_2O 3 eq. in py, rt, 12 h, 75%; (viii) TFA/ CH_2Cl_2 , 50%, rt, 1 h, 90%. Np = *p*-nitrophenyl.

Compound **102a** (**Scheme 29**) was taken through a similar synthesis to the one described in **Scheme 28** to form oxazoles *via* a different method. Compound **102a** was reacted with the alkyne containing acrylate to afford **139** which then underwent a cycloaddition to form **140**. The ester in **140** was hydrolysed, the amine was deprotected

and coupled to a protected amino acid, followed by acetylation and deprotection to give **141**. The synthesised compounds were tested as inhibitors of ACE, ACE2 and carboxypeptidase A (CPA). (**Table 17**).

| No. | Compound | ACE2 K _i (nM) | CPA K _i (nM) | ACE K _i (μM) |
|----------------|--|-----------------------------|----------------------------|----------------------------|
| 128a | Cbz-ProΨ(PO ₂ -CH ₂)Leu-OH | 300 | - | - |
| 128b | Cbz-ProΨ(PO ₂ -CH ₂)Phe-OH | 300 | - | - |
| 128c | Cbz-ProΨ(PO ₂ -CH ₂)Ala-OH | 3000 | - | - |
| 130a | Cbz-LeuΨ(PO ₂ -CH ₂)Phe-OH | >10000 | - | - |
| 130b | Cbz-PheΨ(PO ₂ -CH ₂)Phe-OH | >10000 | - | - |
| 130c | Cbz-AlaΨ(PO ₂ -CH ₂)Phe-OH | 8000 | - | - |
| 131a | LeuΨ(PO ₂ -CH ₂)Phe-OH | >10000 | - | - |
| 131b | PheΨ(PO ₂ -CH ₂)Phe-OH | >10000 | - | - |
| 131c | AlaΨ(PO ₂ -CH ₂)Phe-OH | >10000 | - | - |
| 132 | ProΨ(PO ₂ -CH ₂)Phe-OH | >10000 | - | - |
| 133a | Ac-Ala-ProΨ(PO ₂ -CH ₂)Phe-OH | 7.5 | - | - |
| 133b | Ac-Leu-ProΨ(PO ₂ -CH ₂)Phe-OH | 0.35 | - | - |
| 133bFII | Ac-Leu-ProΨ(PO ₂ -CH ₂)Phe-OH, FII | 0.13 | 0.5 | >10 |
| 133c | Ac-Lys-ProΨ(PO ₂ -CH ₂)Phe-OH | 6.5 | - | - |
| 133d | Ac-Glu-ProΨ(PO ₂ -CH ₂)Phe-OH | 7 | - | - |
| 133e | Ac-Tyr-ProΨ(PO ₂ -CH ₂)Phe-OH | 5.2 | - | - |
| 133f | Ac-Phe-ProΨ(PO ₂ -CH ₂)Phe-OH | 5.2 | - | - |
| 133g | Ac-Val-ProΨ(PO ₂ -CH ₂)Phe-OH | 6.6 | - | - |
| 133h | Ac-His-ProΨ(PO ₂ -CH ₂)Phe-OH | 2.1 | - | - |
| 133hFII | Ac-His-ProΨ(PO ₂ -CH ₂)Phe-OH, FII | 0.7 | 60 | >10 |
| 134a | Ac-His-LeuΨ(PO ₂ -CH ₂)Phe-OH | 920 | 0.3 | - |
| 134b | Ac-Leu-PheΨ(PO ₂ -CH ₂)Phe-OH | 800 | 175 | - |
| 138aFII | Ac-Leu-ProΨ(PO ₂ -CH ₂)Isoxa(Phe)*-OH | 1.25 | 35 | >10 |
| 138bFII | Ac-His-ProΨ(PO ₂ -CH ₂)Isoxa(Phe)*-OH | 0.4 | 1050 | >10 |
| 141FII | Ac-His-LeuΨ(PO ₂ -CH ₂)Isoxa(Phe)*-OH | 220 | >10 μM | - |

Table 17 Inhibition of ACE2, CPA and ACE by phosphinic peptide inhibitors (*Isoxa(Phe): 2-(3-phenyl-isoxazol-5-ylmethyl)-propionic acid).

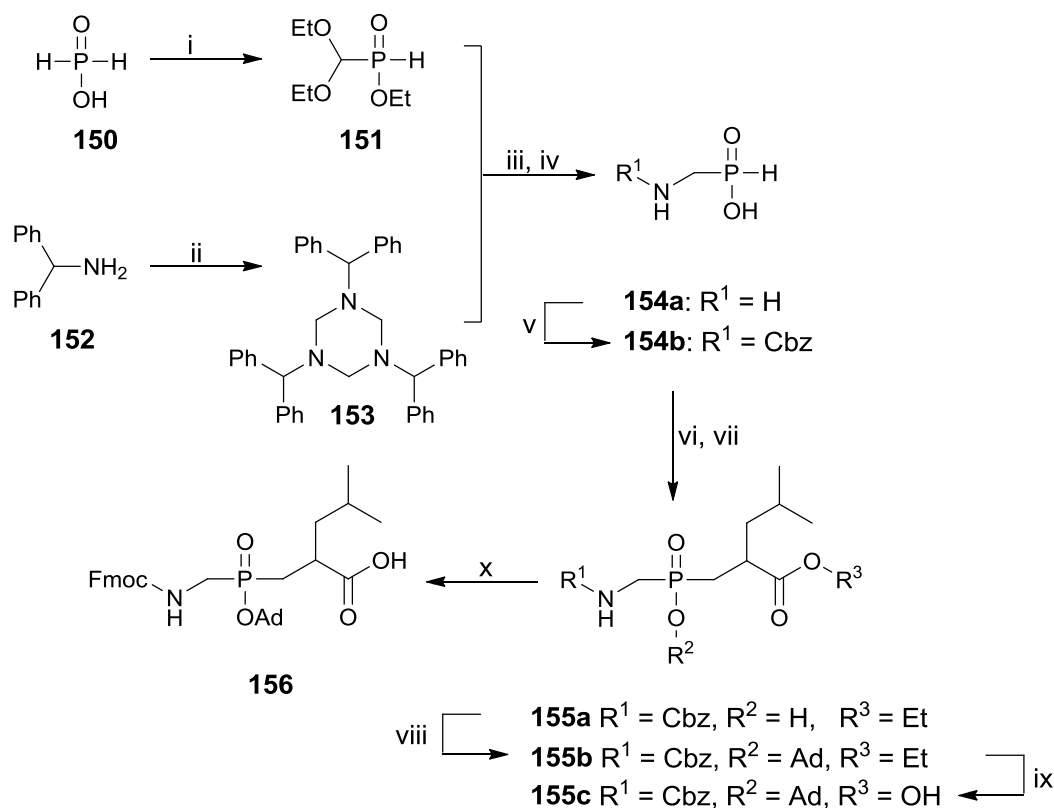


Scheme 30 Reagents and conditions: (i) 20% piperidine/DMF; (ii) FmocGlu(O^tBu)-OH, HOBt, HBTU, NMM; (iii) 20% piperidine/DMF; (iv) acryloyl chloride, Et₃N; (v) bis-(trimethylsilyl)acetamide, DCE, 100 °C; (vi) Pd(PPh₃)₄, CH₃Cl/AcOH/NMM 92.5:2.5:2.5; (vii) 20% piperidine/DMF; (viii) Fmoc-amino acids, HBTU, NMM; (ix) 20% piperidine/DMF; (x) TFA/H₂O 95:5.

Kruger *et al.*³⁸ synthesised phosphinic peptidomimetics for use as inhibitors of the *Staphylococcus aureus* sortase transpeptidase (SrtA) using a solid-supported acrylate.

The synthesis of **149** (Scheme 30) began with attaching two glutamic acid residues to resin **142** to form **143**. Compound **143** was deprotected and reacted with acryloyl chloride to give **144**. Compound **144** was reacted with the activated form of amino phosphinic acid **145** to afford **146**. Compound **146** was deprotected to give **147**. Compound **147** then underwent deprotection and coupling reactions with protected amino acids to build up the peptide chain forming **148**. Compound **148** was finally deprotected and cleaved from the resin to give target compound **149**.

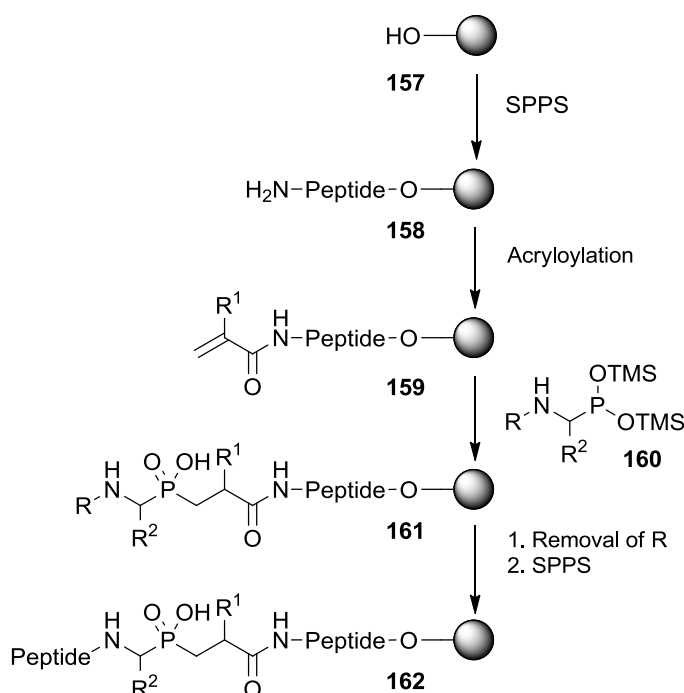
Buchardt *et al.*³⁹ reported the synthesis of diastereomerically impure phosphinic dipeptide analogues as potential MMP-9 inhibitors.



Scheme 31 Reagents and conditions: (i) $CH(OEt)_3$, rt, 55%; (ii) aq. $CH_2=O$, cat. KOH, 90 °C, 87%; (iii) toluene, 100 °C; (iv) 48% aq. HBr, Δ , 73%; (v) CbzCl, K_2CO_3 , H_2O , rt, 72%; (vi) HMDS, 110 °C; (vii) $CH_2=CH(tBu)-CO_2Et$, 90 °C, 96%; (viii) AdBr, Ag_2O , $CHCl_3$, Δ , 89%; (ix) NaOH (EtOH), rt; (x) H_2 , Pd/C, FmocOSu, $NaHCO_3$, MeOH/EtOAc/ H_2O , rt, 65%.

The synthesis of phosphinic peptide **156** (**Scheme 31**) involves multiple steps beginning with formation of two components that are reacted to give **154a**. Phosphinic acid **150** is reacted with trioxymethane to give **151**. Diphenylmethanamine **152** is reacted in the presence of formaldehyde to give triazine **153**. Compounds **151** and **153** are reacted together to form **154a** followed by protection of the amine to give **154b**. Compound **154b** is activated using HMDS then reacted with an acrylate to give **155a**. Compound **155a** was then reacted with bromoadamantane to give **155b**. The ester of **155b** is hydrolysed to give **155c**. The amine in **155c** is deprotected using hydrogenolysis then protected with Fmoc to give **156** that is a suitable building block for peptide synthesis.

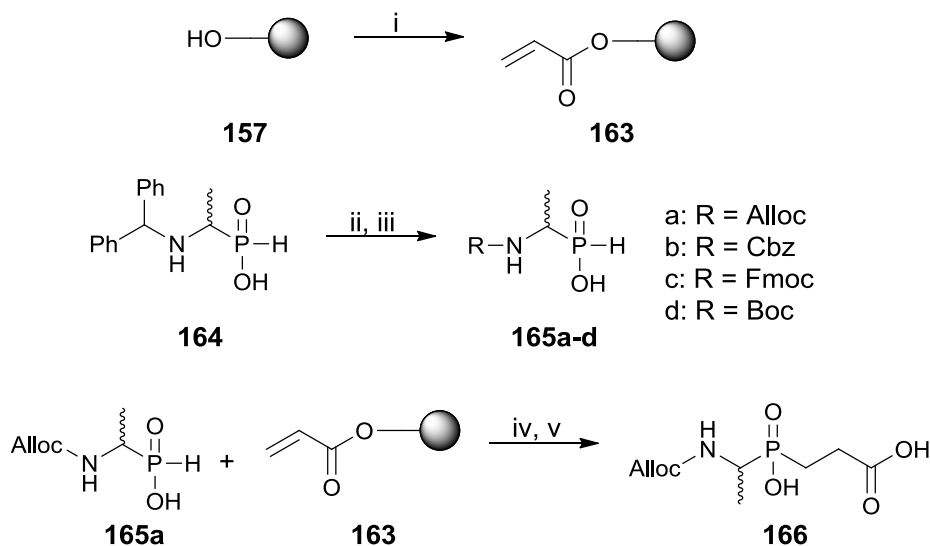
Buchardt *et al.*⁴⁰ also reported a novel methodology for the solid-phase synthesis of phosphinic peptides building on from their previous work (**Scheme 31**).



Scheme 32 Strategy for solid-phase phosphinic peptide synthesis.

The overall strategy for the building of the phosphinic peptide is described in **Scheme 32**. The first step is solid phase peptide synthesis to form peptide **158**. Then the amine is converted into an acrylate **159**, which can react with the phosphinic amino acid **160** to give **161**. After deprotection of the N-protecting group the peptide is extended at the

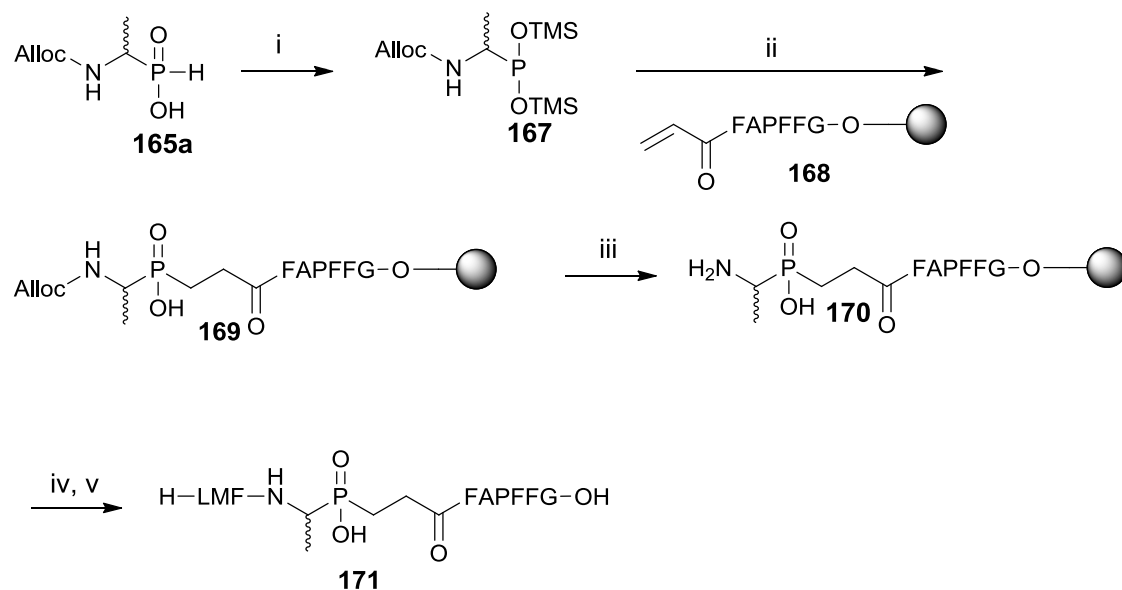
other end of peptide chain in **162**. The actual steps in the synthesis are discussed in the following schemes (**Scheme 33**, **Scheme 34**):



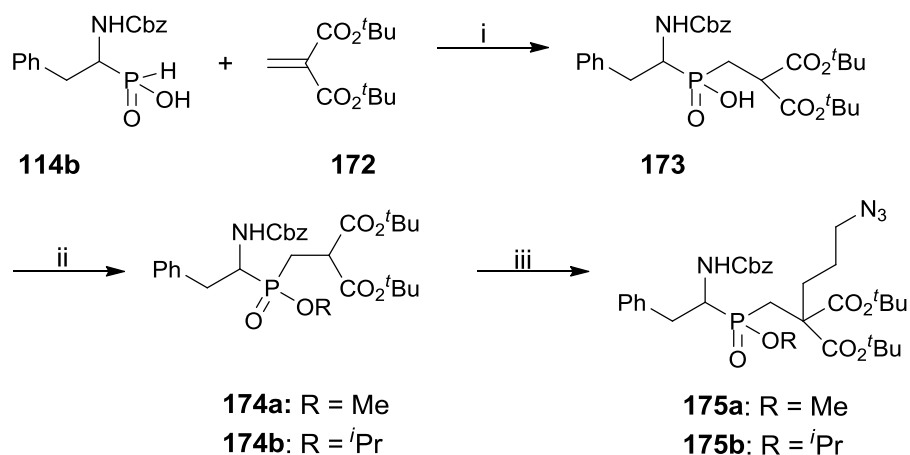
Scheme 33 *Reagents and conditions:* (i) $\text{CH}_2=\text{CHCOCl}$, Et_3N , DMAP; (ii) 48% HBr aq.; (iii) Protection in basic aq. solution (a: AllocCl , b: CbzCl , c: FmocOSu , d: Boc_2O); (iv) BSA (v) NaOH .

Polymer support **157** is converted into acrylate **163**. Compound **164** the alanine analogue 1-aminoethylphosphonic acid is deprotected with hydrobromic acid and protected with various protecting groups under basic conditions to obtain the desired phosphinic amino acids **165a-d**. As an example compound **165a** is reacted with **163** using BSA as an activator, followed by cleavage from the polymer support to give **166**.

Compound **165a** was activated with BSA to give **167**. Compound **167** underwent a Michael addition with **168** to afford **169**. Compound **169** was deprotected using tetrakis(triphenylphosphine)palladium(0) and *N*-ethylmorpholinium acetate (NEM) to give **170**. Compound **170** subsequently underwent further peptide synthesis to give the phosphinic undcapeptide that was cleaved from the resin to give **171**.

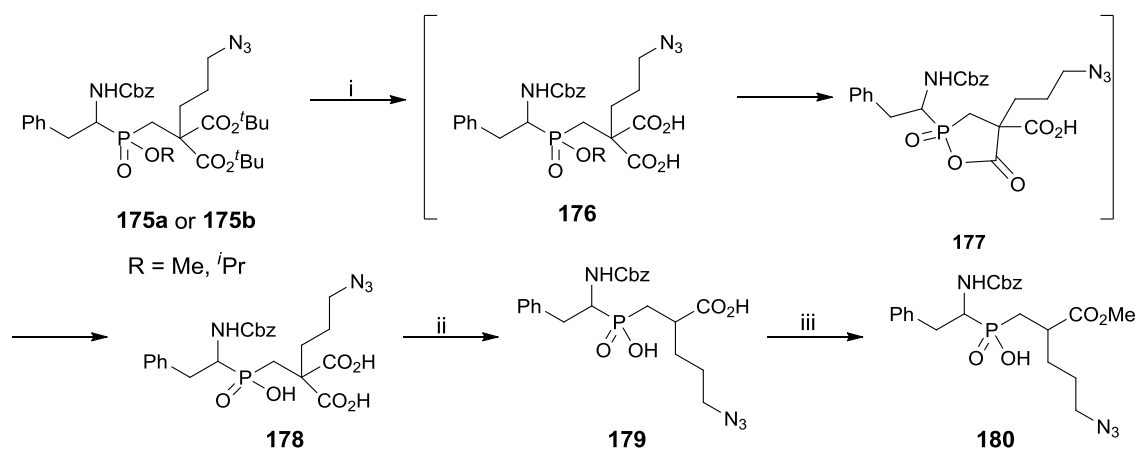


Scheme 34 Reagents and conditions: (i) 3 eq. BSA; (ii) **168**; (iii) $\text{Pd(PPh}_3)_4$, NEM, AcOH; (iv) SPPS; (v) NaOH.



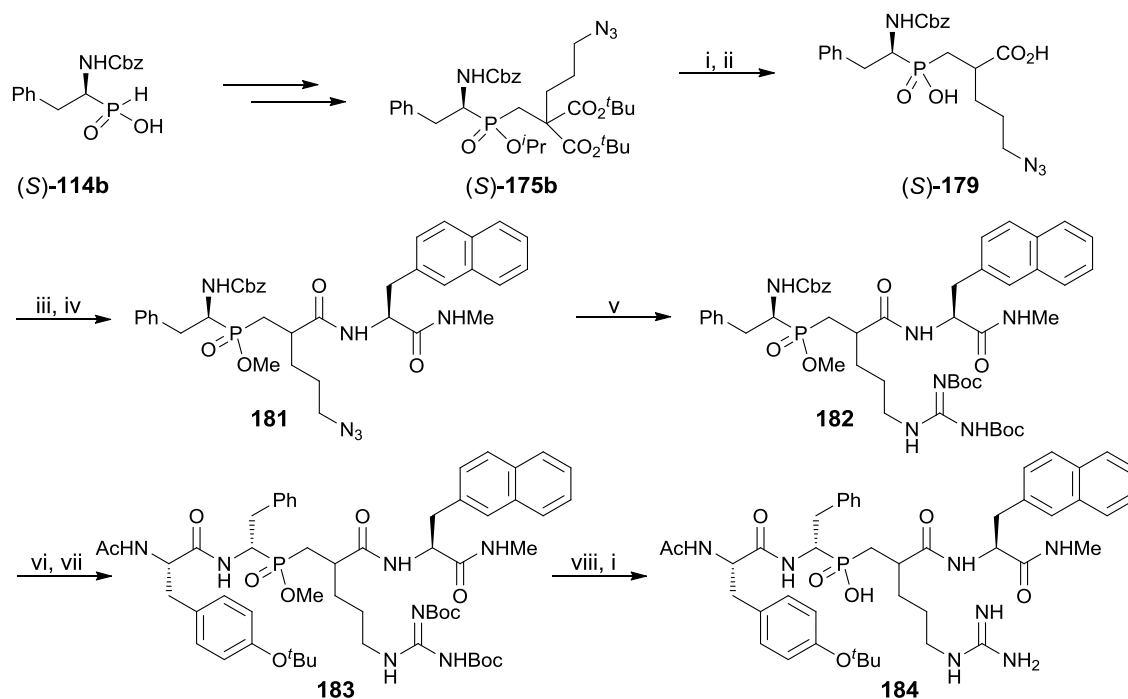
Scheme 35 Reagents and conditions: (i) $^i\text{Pr}_2\text{NEt}$, TMSCl, CH_2Cl_2 ; (ii) CH_2N_2 for **174a** 68%, EDCI, $^i\text{PrOH}$, CH_2Cl_2 for **174b** 72%; (iii) $t\text{-BuOK}$, $\text{I(CH}_2)_3\text{N}_3$, DME, 44% for **175a** 88% for **175b**.

Kende *et al.*⁴¹ reported the synthesis of the phe-arg phosphinic acid dipeptide isostere as part of the construction of polypeptides by a modular method.



Scheme 36 Reagents and conditions: (i) TFA, CH₂Cl₂; (ii) toluene, reflux; (iii) CH₂N₂, 61%.

The first step towards the phosphinic isostere involves synthesis of **175** (Scheme 35). Phosphinic acid **114b** was activated using trimethylsilylchloride (TMSCl) then reacted with di-*tert*-butyl methylene malonate **172** to afford **173**. Compound **173** was then reacted with diazomethane to afford **174a**. Compound **174a** was reacted with azido iodopropane to give **175a**. Alternatively, compound **173** was esterified to give **174b** and then reacted with azido iodopropane to give **175b**.



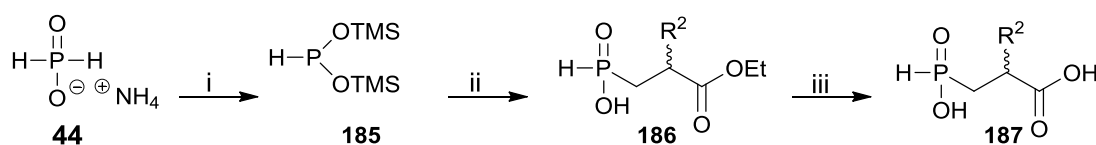
Scheme 37 Reagents and conditions: (i) TFA, CH₂Cl₂; (ii) toluene, reflux; (iii) CDI, (*S*)-2-naphthylalanine-*N*-methylamide, THF; (iv) CH₂N₂, 46%; (v) Lindlar catalyst, H₂, *N,N'*-bis(tert-butoxycarbonyl)-1*H*-pyrazole-1-carboxamide, EtOH, 63%; (vi) 10% Pd/C, HCO₂NH₄, MeOH; (vii) AcNH-Tyr(*t*Bu)-OH, HOBt, EDCI, NMM, THF, 79%; (viii) TMSBr, CH₂Cl₂.

Compounds **175a** or **175b** were treated with TFA which converted them to **178** via postulated intermediates **176** and **177**. Compound **178** was then decarboxylated by refluxing in toluene to give **179**. Compound **179** was then esterified to give **180** to allow for characterisation (**Scheme 36**).

Once the group had synthesised **179**, they adapted the synthesis to a tetrapeptide containing compound **184** (**Scheme 37**). Enantiopure (*S*)-**114b** was reacted as per **Scheme 35** to afford (*S*)-**175b**. Compound (*S*)-**175b** was then treated under the conditions of **Scheme 36** to give (*S*)-**179**. Compound (*S*)-**179** was coupled with (*S*)-2-naphthylalanine-*N*-methanamide then methylated to give **181**. Compound **181** was treated with Lindlar's catalyst and *N,N'*-bis(tert-butoxycarbonyl)-1*H*-pyrazole-1-carboxamide to afford **182**. Compound **182** was deprotected then coupled with a protected tyrosine to give **183**. Compound **183** was finally reacted with TMSBr to give **184**.

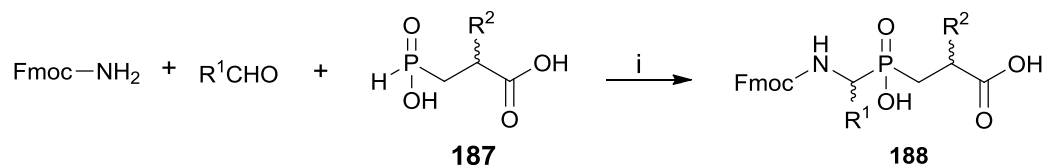
The concept of Michael addition was used in an interesting variation by Matziari *et al.*⁴² as part of their report on a short route to the synthesis of Fmoc-protected phosphinic pseudodipeptide chains as inhibitors of Zinc-metalloproteases. In these examples, hypophosphorous acid is first added to an acrylate. The resulting hydrogen phosphite then undergoes a phospho-aldol reaction.

The first step requires the formation of the P-C bond which is achieved according to **Scheme 38**.



Scheme 38 Reagents and conditions: (i) HMDS, 110 °C, 2 h (Ar); (ii) 0.2 eq. CH₂=C(R²)CO₂Et, 0 °C to rt, 24 h, CH₂Cl₂, yields 75-90%; (iii) NaOH 2M, MeOH, HCl 6M, yields 90-95%.

The first step involves formation of silylated phosphonite **185** via activation of **44** with HMDS to give **185**. Phosphonite **185** is then reacted with various acrylates to give **186**. The final step involves removal of the ethyl ester to give **187**.



R¹ and R² groups are listed in **Table 18**

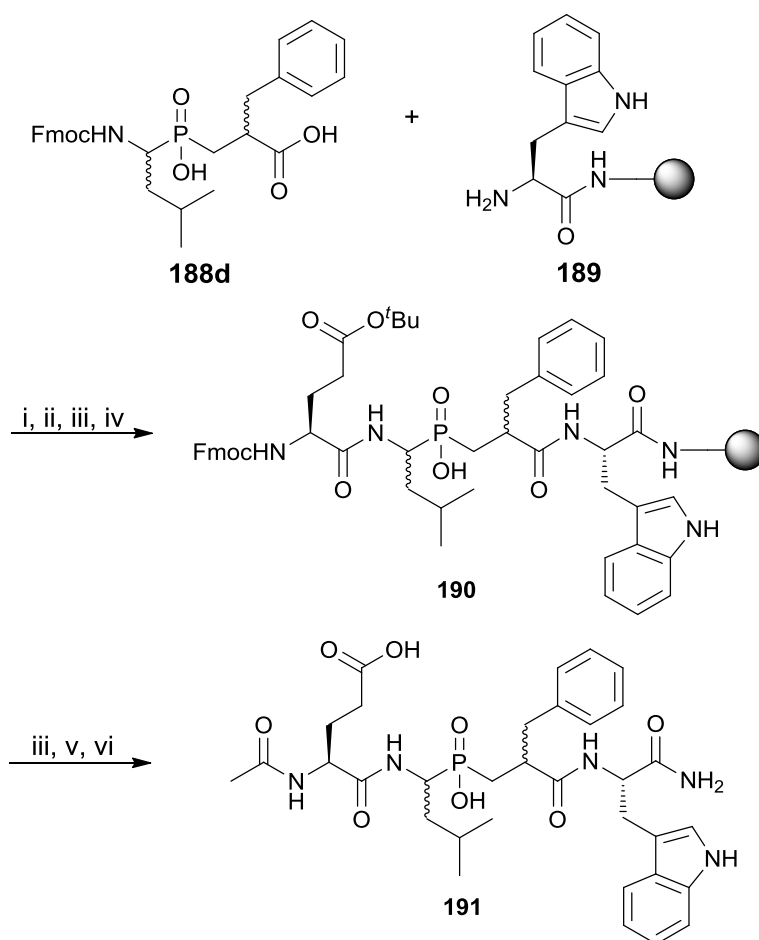
Scheme 39 Reagents and conditions: (i) AcCl/AcOH 5/1, 0 °C to rt, 6 h, 43-73%.

Formation of phosphino amino acid **188** is carried out in a one pot coupling of the three components (**Scheme 39**). Compound **187** is reacted with FmocNH₂ and the desired aldehyde using a mixture of acetyl chloride and acetic acid to catalyse the reaction forming **188** that is suitable for solid phase synthesis. The full details of all phosphinic amino acids synthesised is in the following table (**Table 18**).

| No. | R ¹ | R ² | Yield (%) |
|-------------|---|---|-----------|
| 188a | H | PhCH ₂ | 56 |
| 188b | CH ₃ | PhCH ₂ | 57 |
| 188c | (CH ₃) ₂ CH | PhCH ₂ | 55 |
| 188d | (CH ₃) ₂ CHCH ₂ | PhCH ₂ | 62 |
| 188e | CH ₃ CH ₂ (CH ₃)CH | PhCH ₂ | 61 |
| 188f | CH ₃ O ₂ CCH ₂ CH ₂ | PhCH ₂ | 60 |
| 188g | Ph | PhCH ₂ | 73 |
| 188h | PhCH ₂ OCH ₂ | PhCH ₂ | 67 |
| 188i | 4-imidazole | PhCH ₂ | 69 |
| 188j | CH ₃ | (CH ₃) ₂ CHCH ₂ | 58 |
| 188k | Ph | CH ₃ | 54 |
| 188l | (CH ₃) ₂ CHCH ₂ | H | 42 |

Table 18 Yields and side-chains of compounds **188a-l**.

Once compounds **188a-l** were formed it was possible to extend the peptide chain (**Scheme 40**).



Scheme 40 *Reagents and conditions:* (i) EDC.HCl 4 eq., HOBt 1 eq.; (ii) DIPEA 1.9 eq., CH₂Cl₂, rt. (iii) Piperidine/DMF 20% rt; (iv) FmocGlu(O^{*t*}Bu)Pfp 3 eq., HOBt 3 eq., DMF, rt; (v) AcOH 6 eq., DIC 6 eq., HOBt 6 eq., DMF, rt. (vi) TFA/TIS/H₂O = 95/2.5/2.5, Yield 85%, purity 89%.

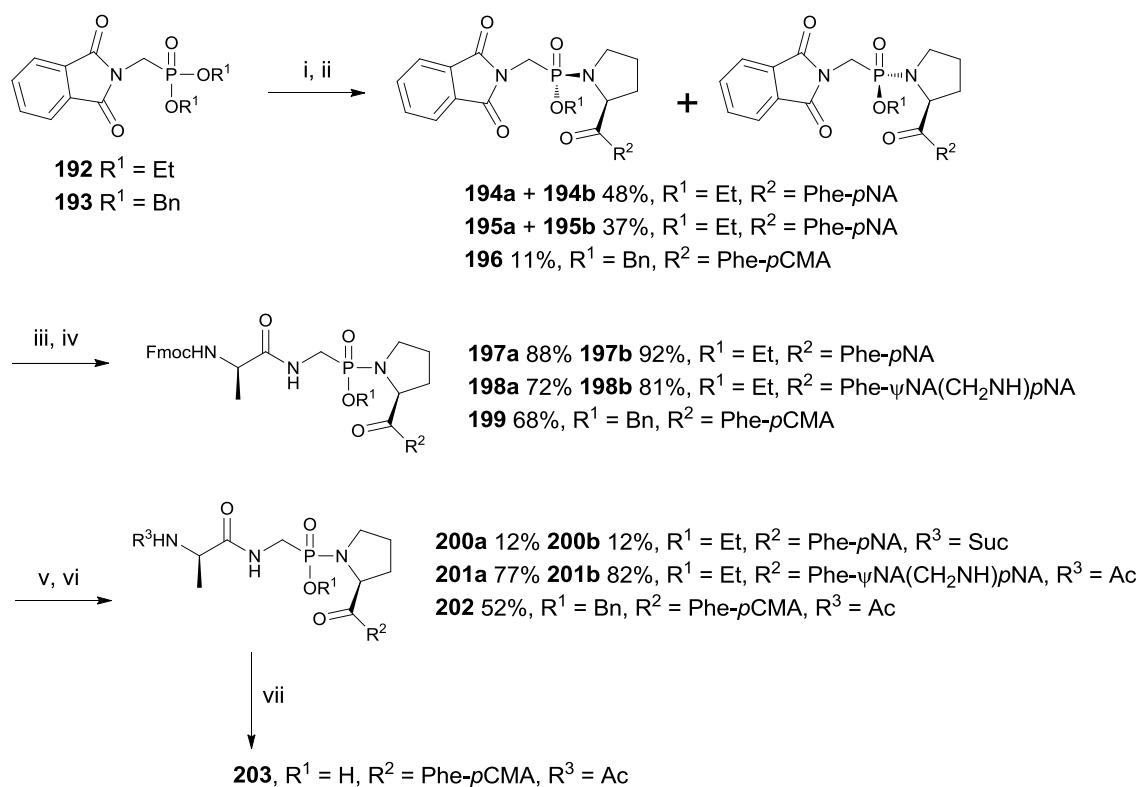
An example of the chain extension is shown in **Scheme 40**. Compound **188d** is coupled to the solid supported tryptophan **189**. The amine is deprotected followed by coupling to another modified amino acid to give **190**. To obtain the final compound the Fmoc group is removed and replaced with an acetate followed by deprotection of the *t*-butyl ester and cleavage from the polymer support to give **191**.

1.4. Reactions involving other methods

In addition to the two most widely used reaction types discussed in sections **1.2** and **1.3**, there are a number of other methods for the synthesis of phosphonopeptides, the scope for which are not yet as wide spread as for the other two. In this section, we will discuss the most recent of these methods.

Generally speaking, direct conversion of phosphonates and phosphinic acids to the corresponding phosphonamide is energetically disfavoured on the account of the strong P-O bond. However, phosphonates and phosphinic acids may be converted to the corresponding phosphoryl chlorides, which can then undergo amidolysis. However, the conditions used is somewhat harsh and therefore, has limited application. Demange *et al.*⁴³ reported the synthesis and evaluation of a number of novel phosphorus containing pseudopeptides against human cyclophilin (hCyp-18). hCyp-18 is associated with control of CD4⁺ T-cells in HIV-1 making it a useful target for anti-HIV-1 therapy.

The synthesis of pseudopeptides **201-203** is outlined in **Scheme 41**. The first step involves activation of phosphonate **192** or **193** with phosphorus pentachloride followed by reaction with substituted prolines to give **194-196**. The phthalyl protecting groups of **194-196** were removed followed by coupling to Fmoc protected alanine to give **197-199**. The Fmoc protecting group of compounds **197-199** were removed using diisopropylamine followed by protection of the free amine with succinic anhydride or acetyl chloride to give **200-202**. Compound **202** was subsequently treated to hydrogenolysis to give **203**.



Scheme 41 Reagents and conditions: (i) PCl_5 , (ii) Pro-R^2 ; (iii) N_2H_4 , (iv) FmocAlaOH , DCC , HOBT ; (v) HN^iPr_2 , (vi) Suc_2O or AcCl ; (vii) H_2 , Pd/C , NaHCO_3 .

The synthesised compounds were derivatives of $\text{Suc-Ala-Ala-Pro-Phe-}p\text{NA}$, where one alanine residue was replaced by $\text{Gly}(\text{PO}_2\text{Et-N})$ to give $\text{Suc-Ala-Gly}(\text{PO}_2\text{Et-N})\text{-Pro-Phe-}p\text{NA}$. Once synthesised the compounds were evaluated for their inhibition of hCyp-18 (Table 19).

| Compound | $K_d \pm \text{SD } (\mu\text{M})$ | $\text{IC}_{50} \pm \text{SD } (\mu\text{M})^*$ |
|---|------------------------------------|---|
| $\text{Suc-Ala-Gly-Pro-Phe-}p\text{NA}$ | 140 ± 10 | 1450 ± 60 |
| $\text{Suc-Ala-Gly}\psi(\text{PO}_2\text{Et-N})\text{Pro-Phe-}p\text{NA}$ 201a | 210 ± 100 | 5400 ± 400 |
| $\text{Suc-Ala-Gly}\psi(\text{PO}_2\text{Et-N})\text{Pro-Phe-}p\text{NA}$ 201b | 20 ± 5 | 15 ± 1 |
| $\text{Ac-Ala-Gly}\psi(\text{PO}_2\text{Et-N})\text{Pro-Phe-}\psi(\text{CH}_2\text{-NH})p\text{NA}$ 202a | 1300 ± 200 | NI @ $500 \mu\text{M}$ |
| $\text{Ac-Ala-Gly}\psi(\text{PO}_2\text{Et-N})\text{Pro-Phe-}\psi(\text{CH}_2\text{-NH})p\text{NA}$ 202b | 200 ± 15 | 30% I @ $500 \mu\text{M}$ |
| $\text{Ac-Ala-Gly}\psi(\text{PO}_2\text{-N})\text{Pro-Phe-}p\text{CMA}$ 203 | 79 ± 4 | NI @ $100 \mu\text{M}$ |
| $\text{Ac-Ala-Gly}\psi(\text{COCON})\text{Pro-Phe-}p\text{NA}$ | 127 ± 7 | 215 ± 40 |
| $\text{Suc-Ala-Ala-Pro-Phe-}p\text{NA}$ | 135 ± 20 | 540 ± 70 |
| $\text{Ac-Ala-Ala-Pro-Phe-}p\text{NA}$ | 145 ± 15 | 640 ± 120 |

Table 19 Inhibition of hCyp-18 by pseudopeptides (* IC_{50} (μM) or % inhibition at given concentration, NI = no inhibition at given concentration).

A rare and unusual method for the synthesis of phosphonopeptides was reported by Van der Donk *et al.*⁴⁴ in the synthesis of antibiotic A53868. During the work, the group found the reported and revised structures for A53868 (**204**⁴⁵ and **205**⁴⁶) were incorrect and the actual structure **206** (**Figure 7**) obtained is very different from the originally reported structures.

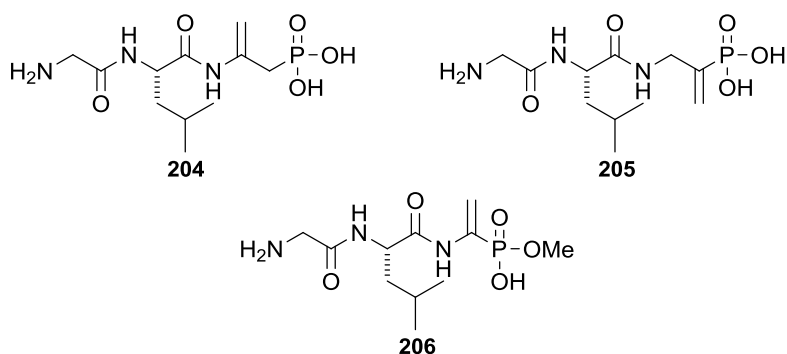
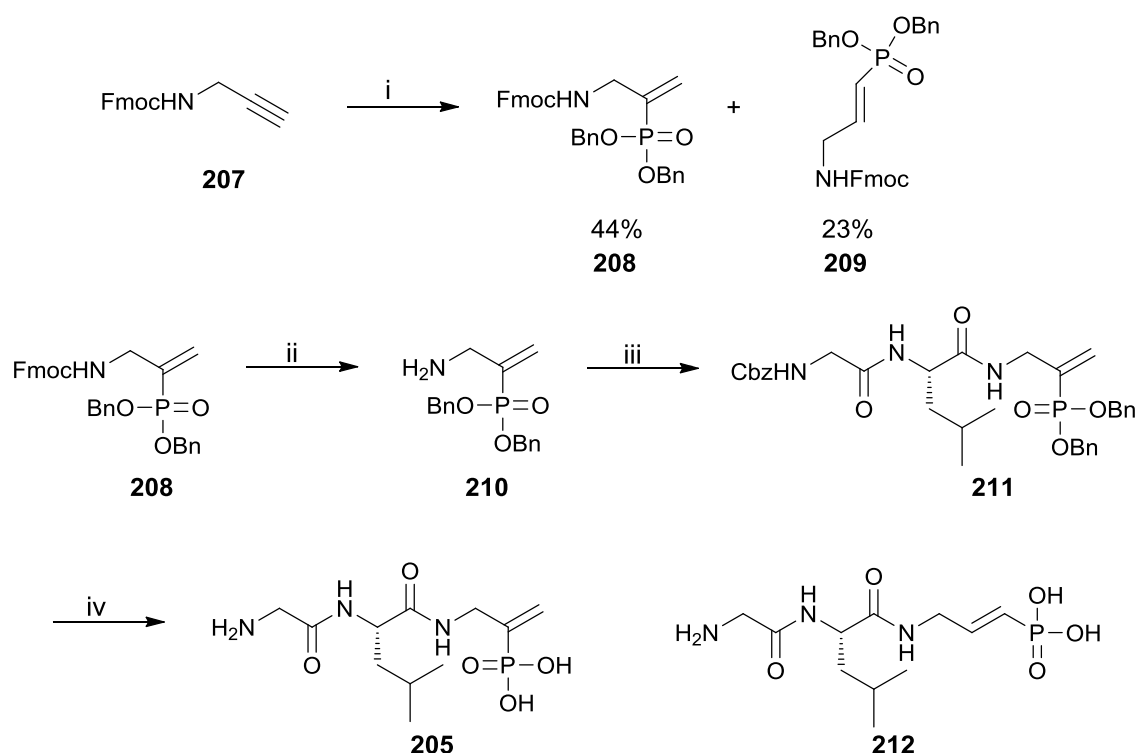


Figure 7 Reported structures of A53868 (**204**, **205**) and actual structure (**206**).



Scheme 42 Reagents and conditions: (i) HP(O)(OBn)_2 , 1 % $[\text{Ni(cod)}_2]$, 8% PPhMe_2 , 4% $\text{Ph}_2\text{P(O)OH}$, THF, 65 °C; (ii) DBU; (iii) Cbz-Gly-Leu, DIC, HOBt; (iv) BBr_3 .

The group first worked towards synthesis of the originally reported structure **205** (**Scheme 42**). Fmoc Protected propargyl amine **207** was treated with dibenzylphosphite

to give vinylphosphonate **208** and its isomer **209**. Compound **208** was deprotected using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to give **210** which was then coupled to Cbz-Gly-Leu to give **211**.

Compound **211** was then treated with boron tribromide to remove the benzyl and Cbz groups to give **205**. Compound **212** was similarly prepared from **209**.

The purified natural product was compared to synthetic compounds **205** and **212** however neither one was found to correspond to the natural product. Detailed nuclear magnetic resonance (NMR) studies were carried out on the natural product which led to structures **213** and **214** (**Figure 8**) being proposed.

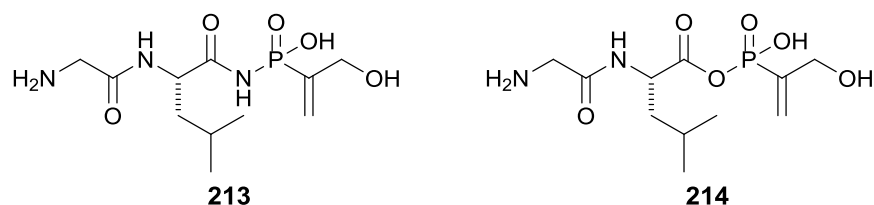
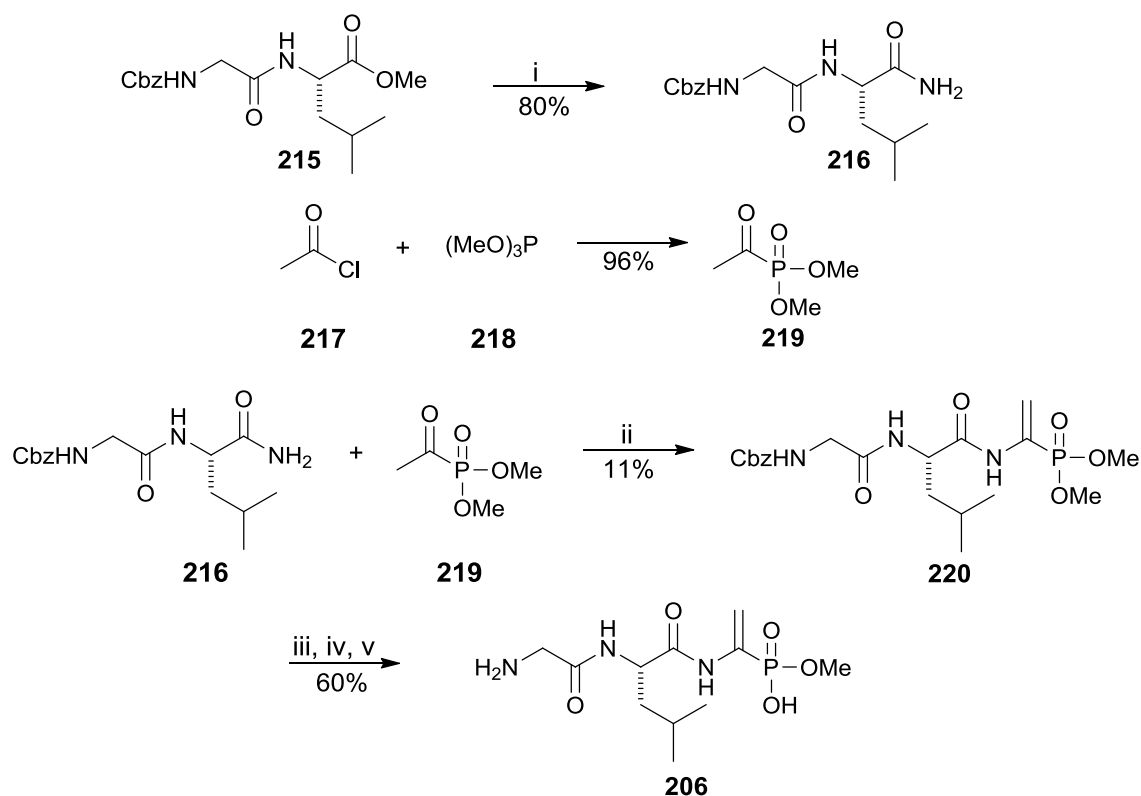


Figure 8 Supplementary proposed structures of A53868.

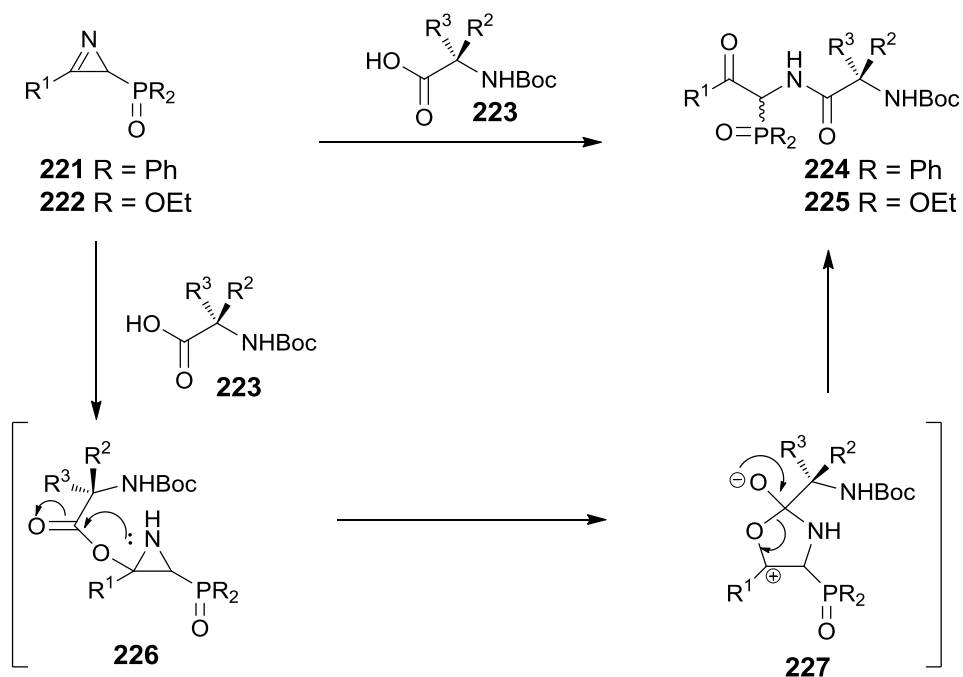
Compounds **213** and **214** were synthesised and studied to see if either structure matched A53868, however neither did. This eventually led the group to propose structure **206**, whose synthesis is outlined below (**Scheme 43**).

Compound **206** was synthesised from fragments **216** and **219**. Dipeptide **216** was synthesised by treating **215** with aqueous ammonia in MeOH to give amide **216**. Formation of α -keto phosphonate **219** was achieved by reacting trimethyl phosphite and acetyl chloride to give **219**. Coupling **216** and **219** was carried out by an acid catalysed condensation to give **220**. Compound **220** was transformed to the carboxy(triethyl)silyl group followed by hydrolysis to give the free amine, followed by basic hydrolysis of one of the esters to give target compound **206**. Compound **206** was proved to be spectroscopically identical to the natural product, and share identical fragmentation patterns in the mass spectrum.



Scheme 43 Reagents and conditions: (i) MeOH/NH₄OH; (ii) TsOH cat., *p*-hydroxyanisole, toluene, reflux; (iii) Et₃SiH, Et₃N, PdCl₂; (iv) NaHCO₃ aq.; (v) 10% NaOH aq.

Palacios *et al.*⁴⁷ synthesised phosphonopeptides as part of the preparation of optically active oxazoles from phosphorylated 2*H*-azirines and N-protected amino acids or peptides (**Scheme 44**).

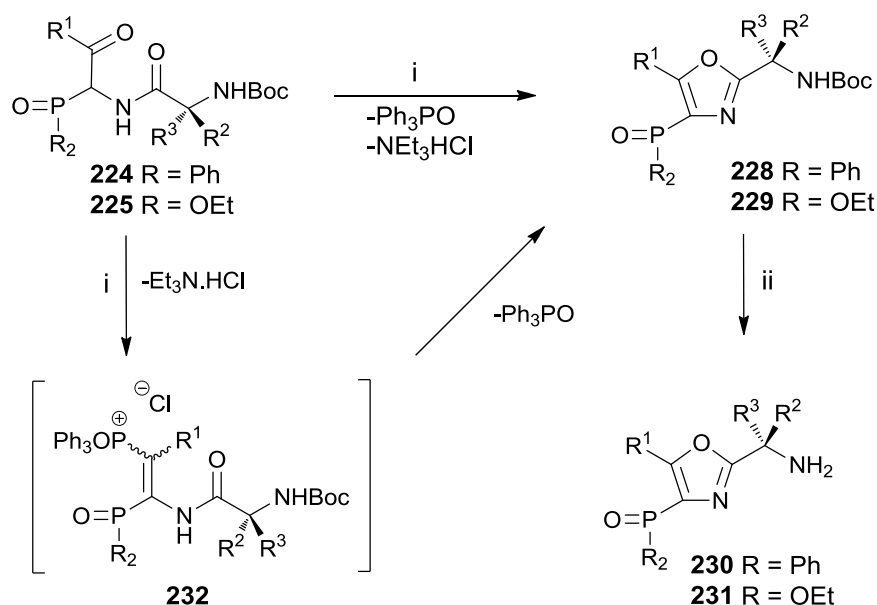


Scheme 44 Synthesis of ketamides.

Reacting 3-methyl-2*H*-aziriny phosphine oxide (e.g. **221** and **222**) with Boc protected amino acids (e.g. **223**) at low temperature forms ketamides that contain a phosphine oxide group **224**, **225**. Formation of **224** is thought to occur due to the mechanism outlined above (**Scheme 44**). Protonation of the azirine, followed by nucleophilic addition of the carboxylate gives **226**. Structure **226** can undergo ring expansion to give zwitterionic oxazolone **227**, which is ring opened to form ketamide **224**. This method of synthesis could be adopted using azirines derived from phosphonates. The full list of compounds synthesised are shown in the following table (**Table 20**).

| No. | R | R ¹ | R ² | R ³ | Yield (%) |
|--------------|--------------------------------|-------------------------------|--------------------|---|-----------|
| 224aa | C ₆ H ₅ | CH ₃ | H | H | 73 |
| 224ab | C ₆ H ₅ | CH ₃ | H | CH ₃ | 65 |
| 224ac | C ₆ H ₅ | CH ₃ | H | CH ₂ OH | 65 |
| 224ad | C ₆ H ₅ | CH ₃ | CH ₃ | H | 58 |
| 224ae | C ₆ H ₅ | CH ₃ | H | CH ₃ | 62 |
| 224af | C ₆ H ₅ | CH ₃ | CH ₂ OH | H | 48 |
| 224ag | C ₆ H ₅ | CH ₃ | H | CH ₂ OH | 66 |
| 224ah | C ₆ H ₅ | CH ₃ | H | CH ₂ C ₆ H ₅ | 56 |
| 225ab | OC ₂ H ₅ | CH ₃ | H | CH ₃ | 68 |
| 225ad | OC ₂ H ₅ | CH ₃ | CH ₃ | H | 71 |
| 225ae | OC ₂ H ₅ | CH ₃ | H | CH ₃ | 66 |
| 225ag | OC ₂ H ₅ | CH ₃ | H | CH ₂ OH | 52 |
| 225bd | OC ₂ H ₅ | C ₂ H ₅ | CH ₃ | H | 72 |
| 225bg | OC ₂ H ₅ | C ₂ H ₅ | H | CH ₂ OH | 50 |

Table 20 Details of R, R¹, R² and R³ for α-ketamides synthesised.

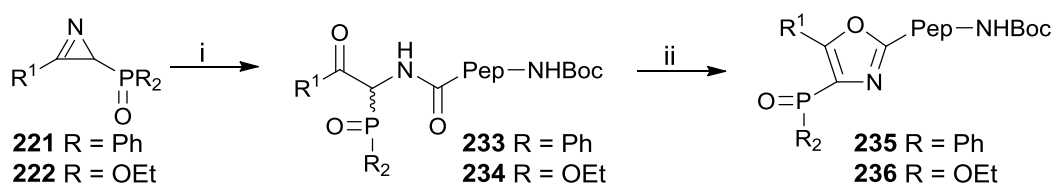


Scheme 45 Reagents and conditions: (i) Ph₃P, C₂Cl₆, Et₃N; (ii) TMSCl, PhOH, CH₂Cl₂.

The synthesised α -ketamides were then converted to phosphorylated oxazoles containing amino alkyl residues (**Scheme 45**). Compounds **224** and **225** were treated with triphenylphosphine and hexachloroethane to give oxazole phosphine oxides **228** and **229**. The proposed mechanism involves deprotonation of ketamides with dichlorotriphenylphosphorane giving enamide intermediate **232**. Compound **232** then undergoes loss of triphenylphosphine oxide and ring closure to give **228** and **229**. Deprotection of **228** and **229** was carried out using TMSCl and phenol for oxazole phosphine oxides, and hydrochloric acid for oxazole phosphonates deprotection to give **230** and **231**. The full list of compounds synthesised are given below (**Table 21**).

| No. | R | R ¹ | R ² | R ³ | Yield (%) |
|--------------|--------------------------------|-------------------------------|-----------------|---|-----------|
| 228aa | C ₆ H ₅ | CH ₃ | H | H | 68 |
| 228ab | C ₆ H ₅ | CH ₃ | H | CH ₃ | 70 |
| 228ad | C ₆ H ₅ | CH ₃ | CH ₃ | H | 72 |
| 228ae | C ₆ H ₅ | CH ₃ | H | CH ₃ | 66 |
| 228ah | C ₆ H ₅ | CH ₃ | H | CH ₂ C ₆ H ₅ | 73 |
| 229ab | OC ₂ H ₅ | CH ₃ | H | CH ₃ | 45 |
| 229ad | OC ₂ H ₅ | CH ₃ | CH ₃ | H | 56 |
| 229ae | OC ₂ H ₅ | CH ₃ | H | CH ₃ | 50 |
| 229bd | OC ₂ H ₅ | C ₂ H ₅ | CH ₃ | H | 46 |
| 230aa | C ₆ H ₅ | CH ₃ | H | H | 81 |
| 230ae | C ₆ H ₅ | CH ₃ | H | CH ₃ | 74 |
| 231ad | OC ₂ H ₅ | CH ₃ | CH ₃ | H | 46 |

Table 21 Details of phosphorylated oxazoles synthesised.



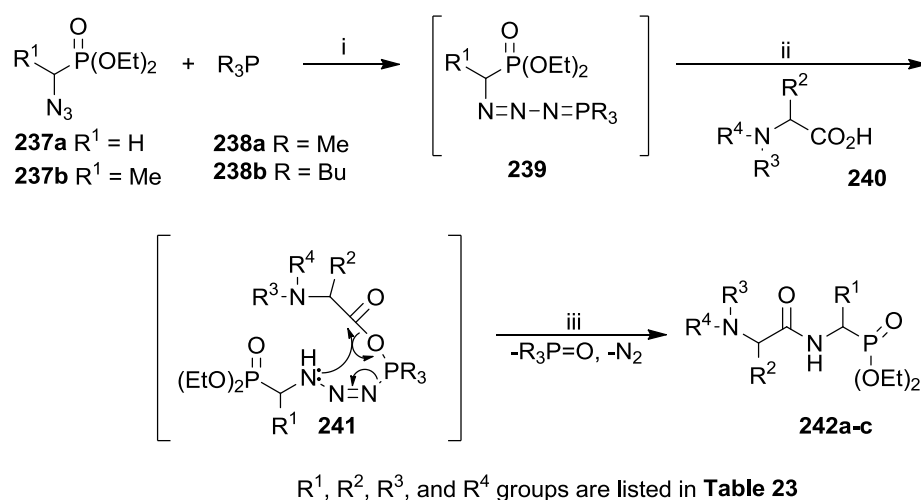
Scheme 46 Reagents and conditions: (i) HO₂C-Pep-NHBoc **233**; (ii) Ph₃P, C₂Cl₆, Et₃N.

Where N-protected peptides are used it is possible to use the process of ring opening azirines (**Scheme 46**). Treating **221** and **222** with peptides formed ketamides **233** and **234**, through a mechanism previously discussed (**Scheme 44**). Treatment of **233** as per **228** (**Scheme 45**), gave **235**. The same method is used to convert **234** to **236**. The full details of compounds synthesised is described in **Table 22**.

| No. | R | R ¹ | Peptide | Yield (%) |
|--------------|--------------------------------|-------------------------------|-----------------|-----------|
| 233aa | C ₆ H ₅ | CH ₃ | (S)-Gly-Phe | 55 |
| 233ab | C ₆ H ₅ | CH ₃ | (S)-Ala-Gly-Gly | 51 |
| 234ba | OC ₂ H ₅ | C ₂ H ₅ | (S)-Gly-Phe | 48 |
| 235aa | C ₆ H ₅ | CH ₃ | (S)-Gly-Phe | 52 |
| 236ba | OC ₂ H ₅ | C ₂ H ₅ | (S)-Gly-Phe | 50 |

Table 22 Details of α -ketamides and phosphorylated oxazoles synthesised.

Sikora *et al.*⁴⁸ have reported on the synthesis of protected phosphonodipeptides containing an N-terminal amino acid using a Staudinger reaction (**Scheme 47**).



Scheme 47 Reagents and conditions: (i) toluene, rt, 1.5 h; (ii) **223** (iii) toluene, 80-85 °C, 3-12 h or rt 24 h - 3 d.

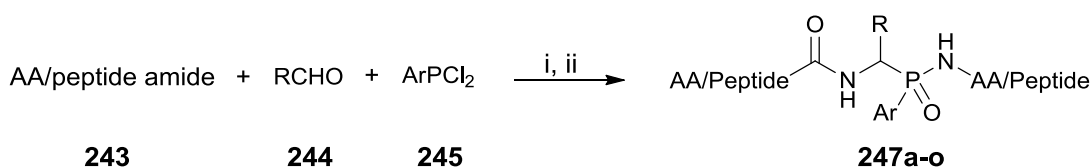
The synthesis of the phosphonodipeptides began by reacting diethyl 1-azidoalkylphosphonate **237a-b** (formed by reacting hydrazoic acid and diethyl 1-hydroxyalkylphosphonates) with phosphine **238a-b** to give the corresponding iminophosphorane **239**. Compound **239** was then reacted with protected amino acid **240** which was converted *via* intermediate salt **241** into the corresponding phosphonodipeptides **242a-c** with the details of substituents given in **Table 23**.

| No. | R ¹ | R ² | R ³ | R ⁴ |
|-------------|----------------|-------------------------------------|----------------|----------------|
| 242a | H | Bn | H | Cbz |
| 242b | H | (CH ₂) ₂ SMe | H | Boc |
| 242c | Me | Me | H | Cbz |

Table 23 Details of R¹, R², R³, and R⁴ groups for phosphonopeptides synthesised.

In an interesting variation to the methods discussed in **section 1.2**, phosphonopeptides can be prepared by the addition to an acyl iminium. Xu *et al.*⁴⁹ reported the synthesis of Cbz protected phosphonopeptides and depsiphosphonopeptides. The synthesis involved a Mannich-type condensation of benzyl carbamate, aldehydes, and dichlorophosphites followed by aminolysis with amino acid ester or alcoholysis with hydroxy acid esters. The aim of the work was to develop a general method to synthesise phosphinopeptides where the aminoalkylphosphinic acid is in the middle of the peptides.

The group carried out lots of investigations into conditions finally achieving success reacting Cbz-glycinamide, benzaldehyde, phenyldichlorophosphine and ethyl glycine to form the phosphonopeptide. The group then extended the method to prepare a series of phosphinopeptides with two phosphinotetrapeptides (**Scheme 48** and **Scheme 49**).



Scheme 48 Reagents and conditions: (i) MeCN; ii) AA/Peptide ester **246**, DIPEA.

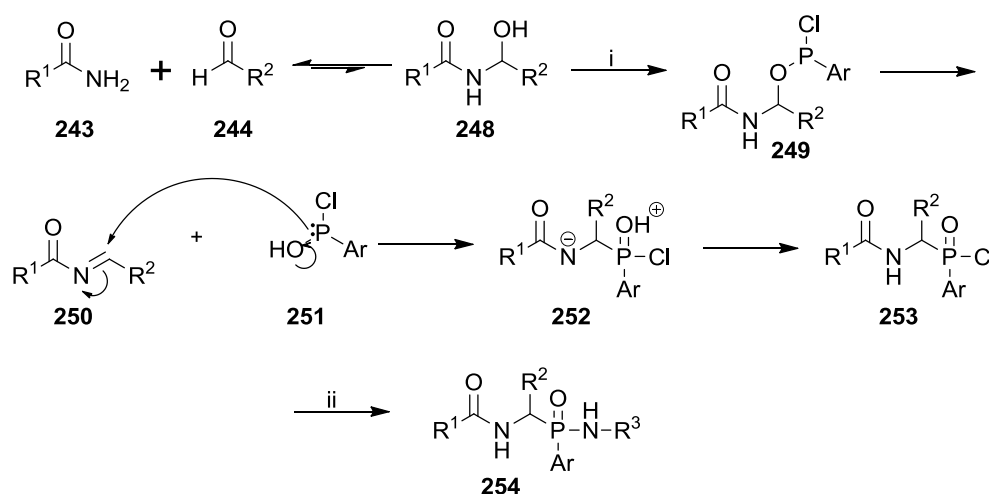
The compounds synthesised according to **Scheme 48** are given in **Table 24**.

The method resulted in formation of oligomeric phosphinopeptides *via* a pseudo four component condensation; first a Mannich-type three component condensation of amide, aldehyde and aryldichlorophosphine, followed by aminolysis with an amino ester or peptide ester. The generality of this method allows the linkage of two peptide sequences with the Mannich-type reaction as the key step, which led to them naming this a Mannich ligation.

| No. | Amide 243 | Aldehyde 244 | Ar 245 | Ester 246 | Yield (%) |
|------|---------------------------------|--------------------|----------------|--------------|--------------|
| 247a | Cbz-Gly-NH ₂ | PhCHO | Ph | GlyOEt | 67 |
| 247b | Cbz-Gly-NH ₂ | PhCHO | <i>p</i> -MePh | GlyOEt | 68 |
| 247c | Cbz-Gly-NH ₂ | PhCHO | <i>p</i> -ClPh | GlyOEt | 71 |
| 247d | Cbz-Gly-NH ₂ | <i>p</i> -MePhCHO | Ph | GlyOEt | 70 |
| 247e | Cbz-Gly-NH ₂ | <i>p</i> -ClPhCHO | Ph | GlyOEt | 65 |
| 247f | Cbz-Gly-NH ₂ | ^t PrCHO | Ph | GlyOEt | 69 |
| 247g | Cbz-Gly-NH ₂ | ⁿ PrCHO | Ph | GlyOEt | 69 |
| 247h | Cbz-(D)/(L)-Ala-NH ₂ | PhCHO | Ph | GlyOEt | 64 |
| 247i | Cbz-β-Ala-NH ₂ | PhCHO | Ph | GlyOEt | 60 |
| 247j | Cbz-Gly-NH ₂ | PhCHO | Ph | (L)-PheOMe | 68 |
| 247k | Cbz-Gly-NH ₂ | PhCHO | Ph | (L)-PheOMe | trace |
| 247l | Cbz-Gly-NH ₂ | PhCHO | Ph | β-Ala OEt | 69 |
| 247m | Cbz-Gly-NH ₂ | PhCHO | Ph | GlyGlyOEt | 51 |
| 247n | Cbz-Gly-NH ₂ | PhCHO | Ph | GlyGlyOEt | 28 |
| 247o | Cbz-GlyGly-NH ₂ | PhCHO | Ph | GlyOEt | 54 |

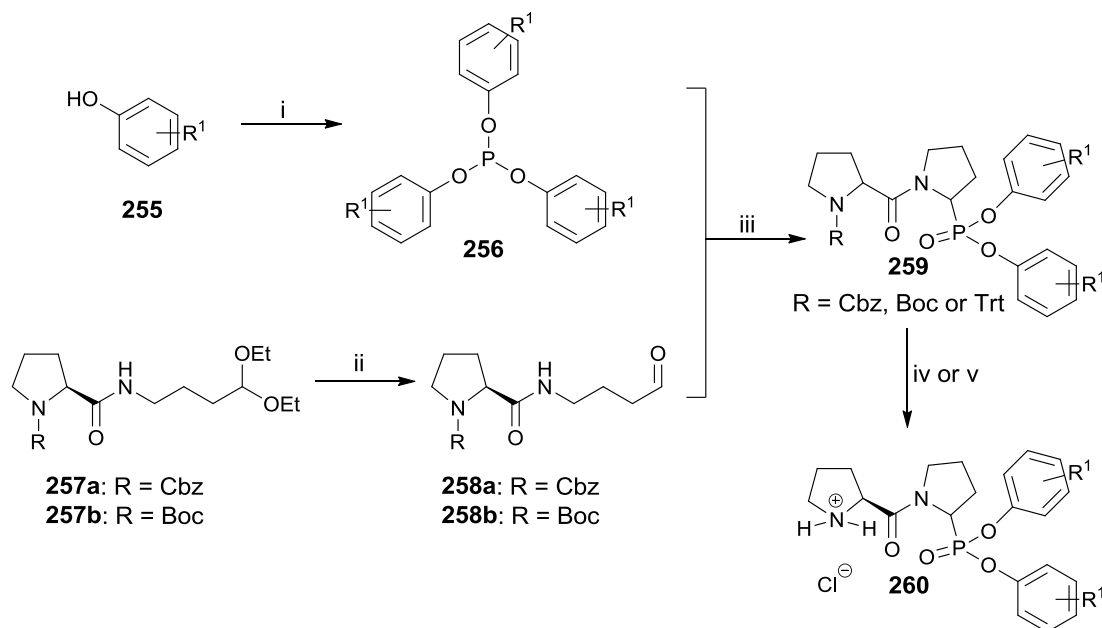
Table 24 Details of components used in synthesis of phosphonopeptides.

The proposed mechanism for the “Mannich Ligation” is outlined below (**Scheme 49**). The first step involves amide **243** attacking aldehyde **244** forming an *N*-acylaminohydrin adduct **248**. Compound **248** reacts with aryldichlorophosphine **245** to give arylchlorophosphite **249**. Compound **249** undergoes elimination to give imine **250** and arylchlorophosphonous acid **251**. Arylchlorophosphonous acid **251** then attacks **250** to give **252** which undergoes proton transfer to give **253**. Compound **253** undergoes aminolysis with an amino ester or peptide **246** to afford **254**.



Scheme 49 Reagents and conditions: (i) ArPCl₂ (**245**); ii) R³NH₂, DIPEA.

A similar method is reported by Belyaev *et al.*⁵⁰ for the synthesis of diaryl phosphonate ester inhibitors of dipeptidyl peptidase IV (DPP IV).

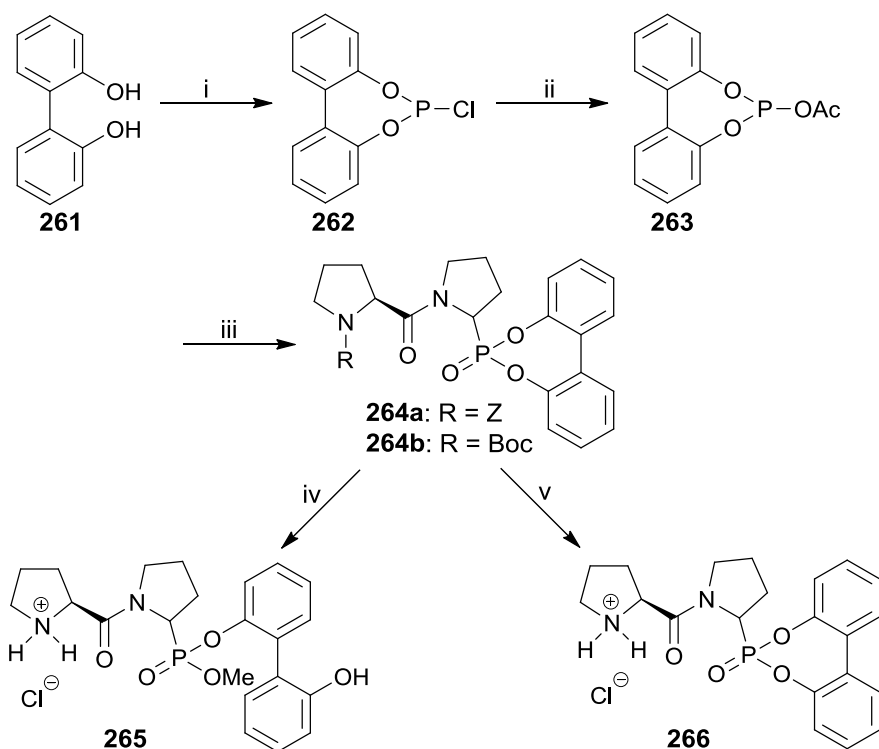


R¹ = a) H, b) 4-OMe, c) 4-OAc (4-OH for **260**), d) 3-NHAc, e) 4-NHAc, f) 4-NHSO₂Me, g) 3-NHCONH₂, h) 4-(N-Bz-Gly-NH), i) 4-(N-Cbz-Gly-NH) [4-(H-Gly-NH) for **260**], j) 4-(N-Cbz-(S)-Ala-NH) [4-(H)-(S)-Ala-NH for **260**], k) 4-[(S)-Pyr-NH], l) 4-[(2S)-MeO₂CCH(NHAc)CH₂], m) 4-CO₂Me, n) 4-(CONHCH₂CO₂Et), o) 4-[CONH(CH₂)₂CO₂Me], p) 4-(CONH(CH₂)₂CH₃).

Scheme 50 Reagents and conditions: (i) PCl₃; (ii) HCl; (iii) AcOH, 90 °C, 2 h; (iv) HCl/EtOAc (1M); (v) H₂, Pd/C.

Synthesis of the diaryl phosphonates **260** (**Scheme 50**) began by reacting phenol **255** with phosphorus trichloride to give triaryl phosphite **256**. Isobutyl chloroformate was used to activate N-protected proline which was then coupled to 4-aminobutyraldehyde diethyl acetal to give **257a-b**. The acetals of **257a-b** were hydrolysed with hydrochloric acid to give **258a-b**. Compounds **258a-b** and **256** were then reacted together to form **259**. The final step involved deprotection of **259** to give **260**.

2,2'-biphenyl derivatives **265** and **266** (**Scheme 51**) were synthesised using a similar strategy to **Scheme 50**. Compound **261** was reacted with phosphorus trichloride to give **262** which was then treated with a mixture of acetic acid and triethylamine to give **263**. Aldehydes **258a-b** were then reacted with **263** to give **264a-b**. Compounds **264a-b** were then deprotected to give **265** and **266**. After synthesis the diaryl phosphonates and biphenyl derivatives were tested as inhibitors of DPP IV (**Table 25** and **Table 26**).



Scheme 51 Reagents and conditions: (i) PCl_3 ; (ii) AcOH , Et_3N ; (iii) **258a-b**, AcOH , 90°C , 2 h; (iv) H_2 , Pd/C , MeOH ; (v) HCl/EtOAc (1M).

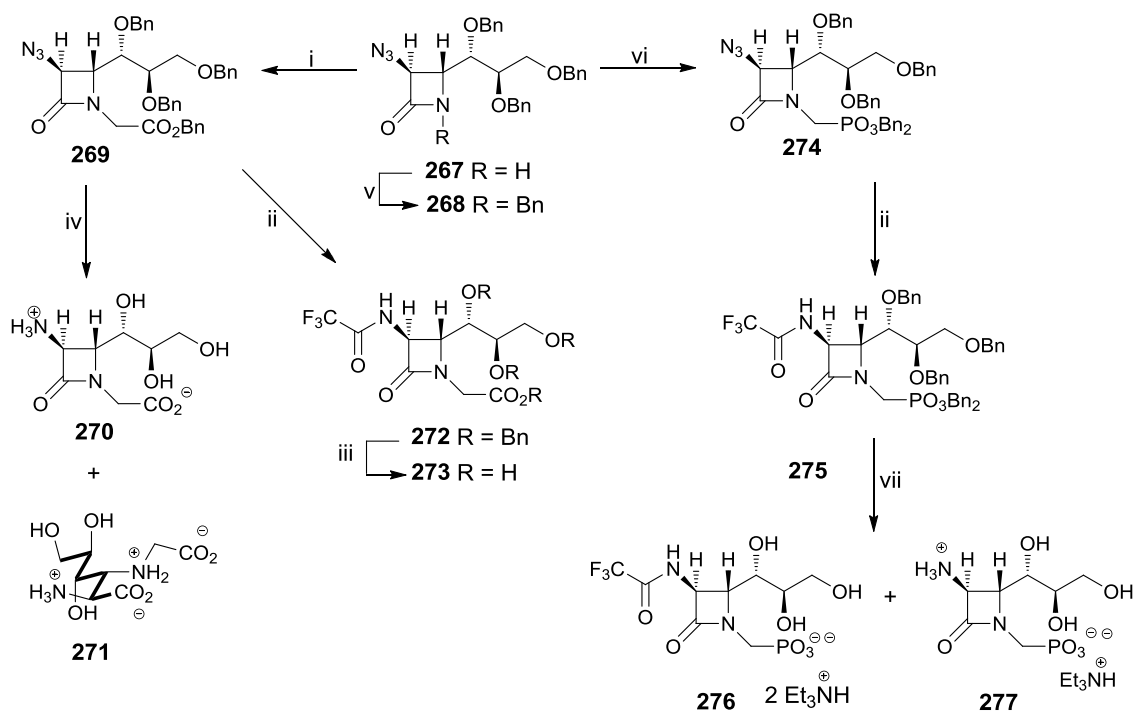
| No. | R^1 | Config at Pro^{P} | IC_{50} (μM) | $k_{\text{calc}}(\text{M}^{-1} \text{s}^{-1})$ | $t_{1/2}$ (min) in plasma |
|-------------|--|--------------------------------------|------------------------------------|--|------------------------------|
| 260a | H | <i>R</i> | 15 ± 3 (n = 3) | 5.1×10^1 | 300 ± 30 |
| 260a | H | <i>S</i> | $> 10^4$ | | |
| 260b | 4-MeO | <i>R,S</i> | 22 ± 11 (n = 4) | 3.5×10^1 | 470 ± 90 |
| 260c | 4-HO | <i>R,S</i> | 190 ± 80 (n = 3) | 4.1 | > 200 |
| 260d | 3-AcNH | <i>R,S</i> | 0.8 ± 0.1 (n = 2) | 9.6×10^2 | 220 ± 30 |
| 260e | 4-AcNH | <i>R,S</i> | 0.4 ± 0.2 (n = 5) | 1.9×10^3 | 320 ± 140 |
| 260f | 4-MeSO ₂ NH | <i>R,S</i> | 0.40 ± 0.02 (n = 2) | 1.9×10^3 | 150 ± 30 |
| 260g | 3-H ₂ NCONH | <i>R,S</i> | 2.3 ± 0.3 (n = 2) | 3.3×10^2 | 210 ± 80 |
| 260h | 4-(N-Bz-Gly-NH) | <i>R,S</i> | 0.7 ± 0.3 (n = 2) | 1.1×10^3 | 93 ± 3 |
| 260i | 4-(H-Gly-NH) | <i>R,S</i> | 0.5 ± 0.1 (n = 2) | 1.5×10^3 | 28 ± 3 |
| 260j | 4-(H-(<i>S</i>)-Ala-NH) | <i>R,S</i> | 0.6 ± 0.2 (n = 2) | 1.3×10^3 | 8 ± 1 |
| 260k | 4-((<i>S</i>)-Pyr-NH) | <i>R,S</i> | 5.0 ± 0.8 (n = 2) | 1.5×10^2 | 170 ± 30 |
| 260l | 4-[(2 <i>S</i>)- MeO ₂ CCH(NHAc)CH ₂] | <i>R,S</i> | 1.4 ± 0.5 (n = 4) | 5.5×10^2 | 190 ± 150 |
| 260m | 4-MeO ₂ C | <i>R,S</i> | 0.016 ± 0.004 (n = 2) | 4.8×10^4 | 19 ± 1 |
| 260n | 4-(EtO ₂ CCH ₂ NHCO) | <i>R,S</i> | 0.023 ± 0.007 (n = 2) | 3.3×10^4 | 35 ± 2 |
| 260o | 4-[MeO ₂ C(CH ₂) ₂ NHCO] | <i>R,S</i> | 0.036 ± 0.006 (n = 4) | 2.1×10^4 | 26 ± 1 |
| 260p | 4-[CH ₃ (CH ₂) ₂ NHCO] | <i>R,S</i> | 0.03 ± 0.01 (n = 2) | 2.6×10^4 | 12 ± 1 |
| 265 | P(OMe)(OC ₆ H ₄ (2-OH-C ₆ H ₄)) | <i>R,S</i> | 47 ± 18 (n = 2) | 1.6×10^1 | 140 ± 80 |
| 266 | P-2,2'-biphenyl | <i>R,S</i> | 31 ± 6 (n = 2) | 2.5×10^1 | 6 ± 1 |

Table 25 Potency and stability of diaryl phosphonate and biphenyl derivative DPP IV inhibitors.

| No. | R ¹ | PEP | | DPP II | | MAAP | | elastase | |
|------|--|------------------|------|------------------|------|------------------|------|------------------|------|
| | | IC ₅₀ | 1 mM | IC ₅₀ | 1 mM | IC ₅₀ | 1 mM | IC ₅₀ | 1 mM |
| 260a | H (R at carbon next to P) | 107 | 71 | 102 | 73 | 100 | 83 | 100 | 82 |
| 260d | 3-AcNH | 89 | 50 | - | - | - | - | - | - |
| 260e | 4-AcNH | 96 | 60 | 100 | 83 | 99 | 96 | 106 | 105 |
| 260f | 4-MeSO ₂ NH | 73 | 4 | - | - | - | - | - | - |
| 260h | 4-(N-Bz-Gly-NH) | 92 | 32 | - | - | - | - | - | - |
| 260j | 4-(H-(S)-Ala-NH) | 88 | 47 | - | - | - | - | - | - |
| 260l | 4-[(2S)-MeO ₂ CCH(NHAc)CH ₂] | 102 | 35 | - | - | - | - | - | - |
| 260m | 4-MeO ₂ C | 73 | 4 | - | - | - | - | - | - |
| 260n | 4-(EtO ₂ CCH ₂ NHCO) | 85 | 1 | 78 | 66 | 104 | 94 | 102 | 6 |
| 260o | 4-[MeO ₂ C(CH ₂) ₂ NHCO] | 83 | 0 | - | - | - | - | - | - |
| 260p | 4-[CH ₃ (CH ₂) ₂ NHCO] | 97 | 5 | - | - | - | - | - | - |

Table 26 Specificity of diaryl phosphonate inhibitors for DPP IV / CD26.

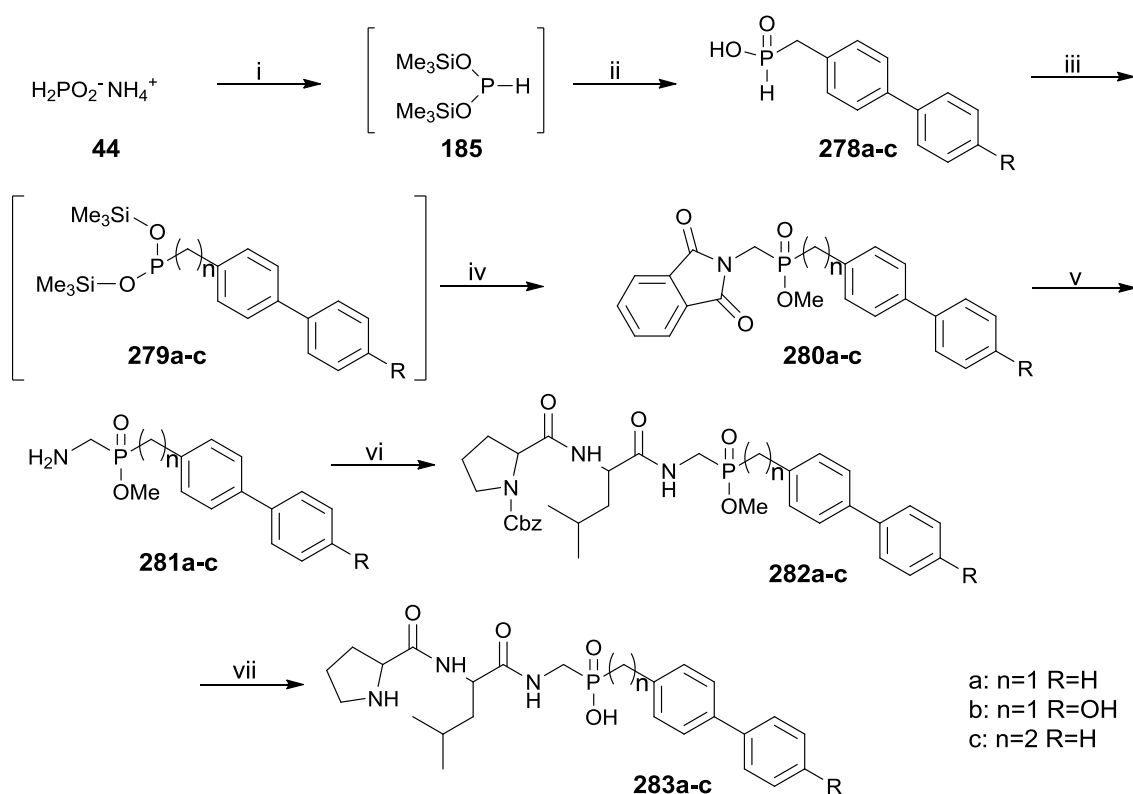
Nucleophilic displacement has also found an application in the synthesis of phosphonopeptides, although its generality is questionable due to the very strict steric requirement in these reactions.



Scheme 52 Reagents and conditions: (i) Ag₂O, BrCH₂CO₂Bn, 1,3-dimethylimidazolidin-2-one; 77%; (ii) (CF₃CO)₂O, PPh₃, THF; DPP, H₂O; 93%; (iii) 20% Pd(OH)₂/C, 7 bar H₂, MeOH/H₂O 5:1; 56%; (iv) 20% Pd(OH)₂/C, 7 bar H₂, ^tBuOH/H₂O 4:1; 54% of **271/272**/Et₃N 2:3:3; (v) ClCH₂PO₃Bn₂, KF/Al₂O₃, K₂CO₃, Bu₄NI, MeCN; 88%; (vi) NaH, 12-crown-4, 1,3-dimethylimidazolidin-2-one/THF, then dibenzyl (triflyloxymethyl)phosphonate; 55%; (vii) Pd(OH)₂/C, 6.5 bar H₂, ^tBuOH/0.1M Et₃NH⁺HCO₃⁻ 3:1; 40% of **277**, 14% of **278**.

Storz *et al.*⁵¹ reported the synthesis of β -lactams which involved the use of phosphorus containing peptides as part of the multi-step synthesis (**Scheme 52**).

β -lactam **267** was N-alkylated using benzyl bromoacetate to give **269**. Compound **269** underwent reductive trifluoroacetylation to give **272**. Deprotection of **272** using hydrogenolysis gave **273**. Hydrogenolysis of azido lactam **269** gave β -lactam **270** and diamino dicarboxylic acid **271** through hydrolysis of **270**. β -lactam **267** was also N-alkylated using dibenzyl (chloromethyl)phosphonate to give **268**. Compound **267** was N-alkylated using dibenzyl (triflyloxymethyl)phosphonate to give **274**. Compound **274** was subjected to reductive trifluoroacetylation as per **269** to give **275**. Hydrogenolysis of **275** was carried out to afford **277**.



Scheme 53 *Reagents and conditions:* (i) HMDS, 110 °C, 1–2 h; (ii) CH₂Cl₂ reflux, 12 h, 58–70% yield; (iii) (Me₃Si)₂NH, CH₂Cl₂, 0 °C, 1 h; (iv) phthalimidomethyl bromide, CH₂Cl₂, reflux, 12 h, and phosphinate methylation after quenching: CH₂N₂, 0 °C, toluene/MeOH, 49–57%; (v) H₂N–NH₂, EtOH, rt, 18 h, 70–78% yield; (vi) Cbz-Pro-Leu-OH, DCC, HOBT, THF, 0 °C to rt, 15 h, 67–80%; (vii) BTSA, CH₂Cl₂, rt, 1 h; TMSI, CH₂Cl₂, -20 °C to rt, 3 h, 72–83%.

In a similar method, Bianchini *et al.*⁵² designed, modelled, synthesised and biologically evaluated three peptidomimetic phosphinates as inhibitors for matrix metalloproteinases MMP-2 and MMP-8. The synthesis of the peptidomimetic phosphinates is described in **Scheme 53**.

The first reaction of the synthesis is as **Scheme 38**, forming compound **185** from **44**, which is then reacted with a number of biphenylalkyl iodides to form **278a-c**. Compounds **278a-c** are treated with bis(trimethylsilyl)amine (BTSA) to give **279a-c** which are immediately treated with phthalimidomethyl bromide followed by methylation to give **280a-c**. Deprotection of **280a-c** using hydrazine afforded **281a-c** which was then coupled with Cbz-Pro-Leu-OH to give **282a-c**. The final step involved deprotection of the Cbz group and hydrolysis of the methyl ester of **282** using BTSA and TMSI respectively to give compounds **283a-c**.

After their synthesis the peptidomimetics were tested for activity against MMP 2 and 8 (**Table 27**). The compounds were shown to have IC₅₀ values in the micromolar range, one of which was a fairly selective MMP-2 inhibitor.

| No. | IC ₅₀ (μM) | |
|-------------|-----------------------|-------|
| | MMP-2 | MMP-8 |
| 283a | 1.7 | 5.1 |
| 283b | 1.4 | 17.6 |
| 283c | 48 | 44.5 |

Table 27 Inhibition of MMP 2 and 8 by peptidomimetic phosphinates.

1.5. Conclusions

One of the most popular methods to form phosphonopeptides is to ligate an existing α -aminophosphonic moiety to the carboxy terminus of a peptide. This method has the dual advantage of being both general and reliable. There are already a number of methods for the synthesis of α -aminophosphonates (including a few enantioselective ones^{53,54,55}). In addition there are a large number of methods and reagents for the formation of peptide bonds. The method has some major drawbacks in that it is best suited to the synthesis of phosphonopeptides with a P-residue at the carboxy end.

Michael addition of a phosphinic/phosphinous acid to an appropriate α -substituted acrylate is the most common and practical route to phosphonopeptides in which the amide linkage is replaced by a P(O)-CH₂ moiety. However, one future direction in this arena could be the development of enantioselective addition of phosphinic/phosphinous acids to α -substituted acrylates.

In addition to the two above general routes, a number of other syntheses are reported although their scope is yet to be demonstrated.

2. Chapter Two: Results and Discussion

2.1. Introduction and Objectives

The concept of phosphonic and phosphinic acids as structural mimics of carboxylic acids has been known for nearly 40 years⁵⁶. The use of these pseudopeptides in which one amino acid residue is replaced by its phosphonic analogue (commonly referred to as phosphonopeptides) represents one of the most straightforward means of producing inhibitors or antagonists to the proteins that bind to that true peptide. Therefore, small pseudopeptides containing a phosphonyl moiety in place of the carboxyl moiety have found extensive medicinal applications.⁵⁷ In the previous chapter, the synthesis and some of the biological properties of these pseudopeptides has been described.

However, despite the prevalence of phosphonopeptides, no study of the suprastructure or conformational preferences of oligomeric α -amino and α -hydroxy phosphonic/phosphinic acids has been conducted yet. This is in spite of the fact that the incorporation of a tetrahedral phosphonic analogue into a peptide chain would be expected to profoundly influence its conformational properties, and thus have a bearing on its intended biological activity. One reason for this could be that methods for the synthesis of phosphonopeptides are quite limited and heavily focus on those that give access to pseudopeptides with the phosphonic residue at either the N or the C terminus of the peptide.

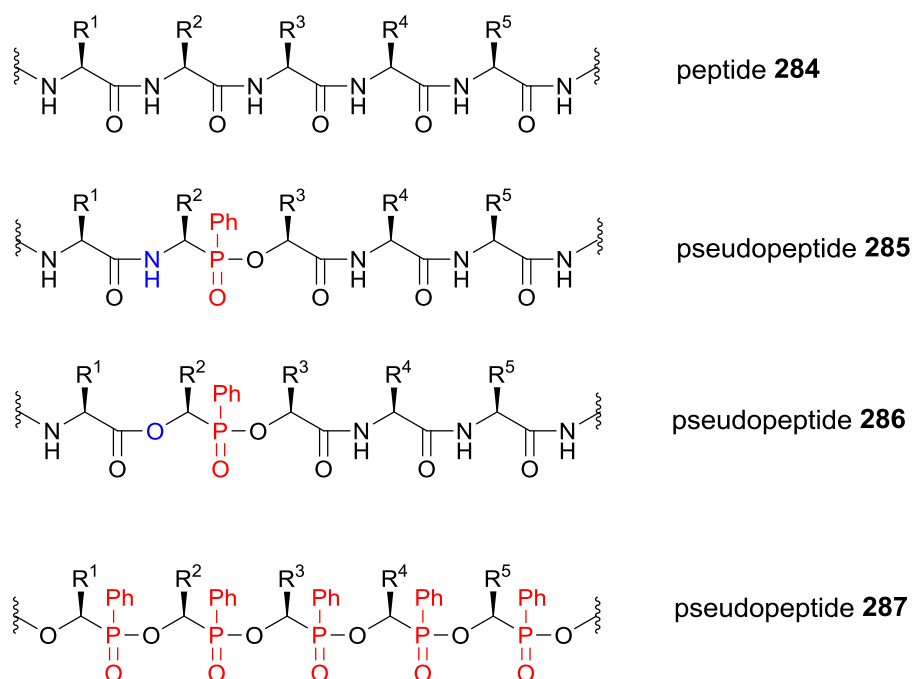
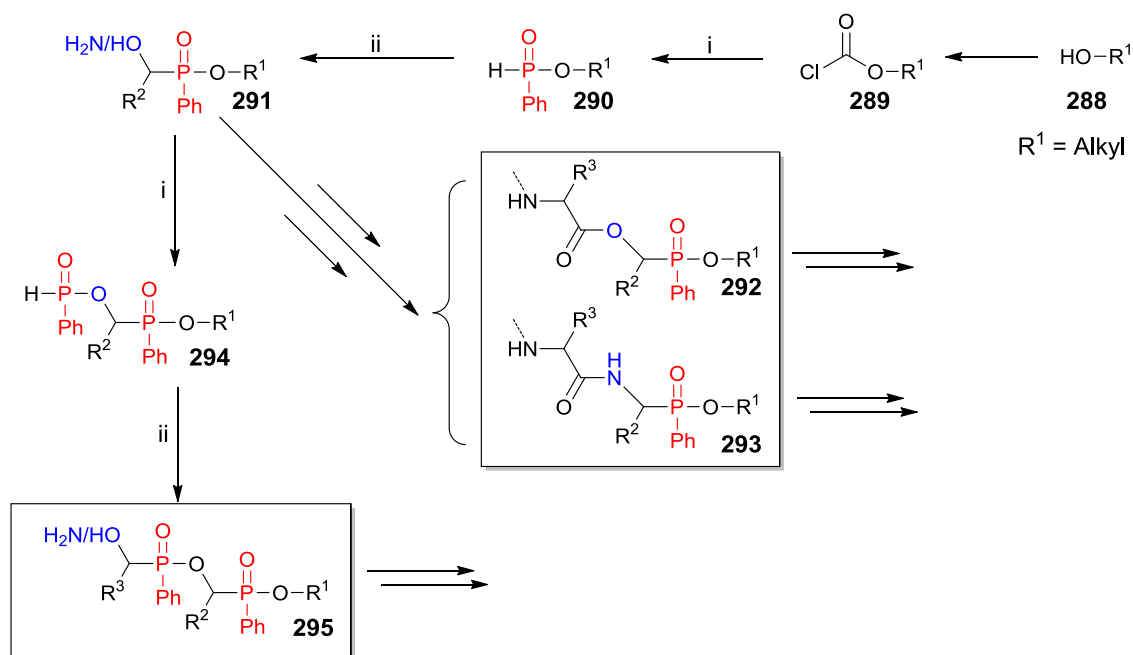


Figure 9 Comparison of peptide and pseudopeptide structures.

In this project, the aim was to investigate the synthesis, and study the conformations of pseudopeptides containing one or more α -amino or α -hydroxy (phenyl)phosphinic acid residues in place of the α -amino carboxylic acid residues (pseudopeptides **285** and **286**, **Figure 9**). In addition we also investigated a method for the synthesis of oligomeric α -hydroxy (phenyl)phosphinates and some conformational preferences of their dimers (pseudopeptide **287**, **Figure 9**). The synthesised pseudopeptides can be directly compared to generalised peptide structure **284** (**Figure 9**).

2.1.1. Synthetic strategy

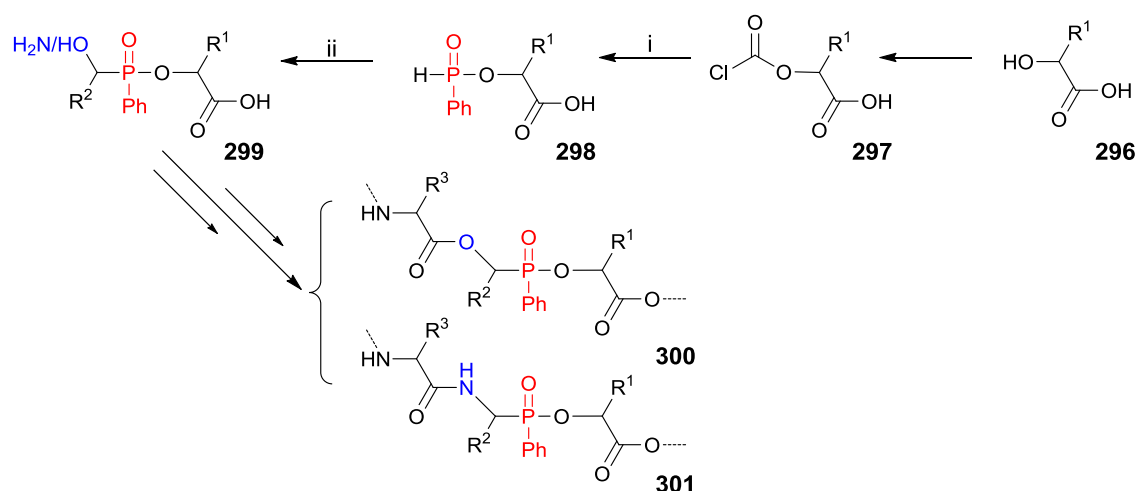
The syntheses were carried out according to the following routes (**Scheme 54**), (**Scheme 55**), depending on the position of the phosphinic acid residue within the chain and the nature of alcohol R¹OH:



Scheme 54 (i) Hewitt reaction; (ii) phospho-aldol reaction.

The first step involves conversion of an alcohol ($R^1\text{OH}$) **288** to the corresponding chloroformate **289**. To prepare phosphonopeptides with a phosphinic residue at the C terminus, the alcohols ($R^1\text{OH}$) can be a simple alcohol such as methanol, ethanol or benzyl alcohol, affording the corresponding phosphinates. The chloroformates are then treated under Hewitt reaction conditions (Page 88)⁵⁸ to give the corresponding phenylphosphinate **290**. Addition of the phenylphosphinate to either aldehydes or imines in a phospho-aldol reaction would then afford the corresponding α -hydroxy or α -amino (phenyl)phosphinate respectively **291**. The hydroxyl or amino functions of the α -hydroxy or α -amino (phenyl)phosphinate product can then be further functionalised **292**, **293** and the pseudopeptide chain can thus be extended from the N-terminus (**Scheme 54**). The hydrolysis or hydrogenolysis of the $R^1\text{O}$ ester would then unmask the phosphinic acid. Alternatively, once an α -hydroxy (phenyl)phosphinate is formed, it can re-enter the synthetic cycle as an alcohol. Thus, through sequential Hewitt **294** and phospho-aldol reactions **295** it is possible to go from monomers to dimers, trimers etc (**Scheme 54**).

To prepare phosphonopeptides with a phosphinic residue at the middle of the chain, the alcohol can be an α -hydroxy carboxylate **296**. As previously formation of chloroformate **297** followed by the Hewitt reaction affords **298**, which can then be used in a phospho-aldol reaction. As before, the hydroxyl or amino functions **299** generated after the addition to aldehydes and imines can be used to further extend the chain at the N-terminus **300**, **301**. Furthermore, however, the carboxyl function in the α -hydroxy acid starter unit can undergo the same chain extension at the C-terminus (**Scheme 55**).



Scheme 55 (i) Hewitt reaction; (ii) phospho-aldol reaction.

The key advantage of this methodology is that it is general. The R^1 (**Scheme 54**) group can range from simple (methyl, ethyl, or benzyl) to complex (α -hydroxy carboxylate). The phospho-aldol reaction is a very general reaction and a wide range of aldehydes or imines can be used to give diversity in the R^2 group.

After synthesis of the compounds, it was planned to use X-ray, computational and NMR methods to ascertain what structural motifs might be present within the structures formed. In order to be able to correlate the results from these pseudopeptides with those of true peptides, a brief description of some of the structural motifs observed in peptides is provided.

2.1.2. Structural motifs observed in peptides⁵⁹

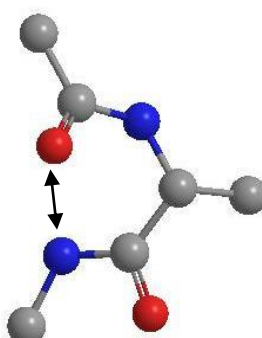
Peptides tend to adopt secondary structures that support their ability to recognise and interact with other molecules. There are three main motifs in the secondary structure of proteins: sheets, turns and helices.

Sheets: This type of secondary structure contains β -sheets (β -pleated sheets). These are formed from beta strands that align side by side and are connected by hydrogen bonds to form a twisted, pleated sheet.

Turns: This is a class of secondary structure that is found within globular proteins that allow for the reversal of the direction of the polypeptide chain. Turns are often situated on or near to sites of antibody recognition, phosphorylation, and glycosylation, so it is likely that they play more than a structural feature.

Turns can be subdivided into four types:

Gamma turn: This is a turn that happens due to hydrogen bonding between the C=O of one residue and the NH of the $i+1$ residue, giving a “folding” pattern due to intramolecular hydrogen bonding (302, **Figure 10**). The gamma turn is less common when compared with the other types of turn.



302

Figure 10 Diagram of a gamma turn.

Type I turn: This type of turn occurs 2-3 times more frequently than the related type II. This turn type has a hydrogen bond between the carbonyl of the first residue and the NH of the $i+2$ residue. In other words, this turn contains a hydrogen bond over three residues (**303, Figure 11**). The mirror image type I' is rare but is preferred in β -hairpins. There are varying preferences for amino acids, with Pro not preferred in the $i+2$ position due to the presence of the secondary amine.

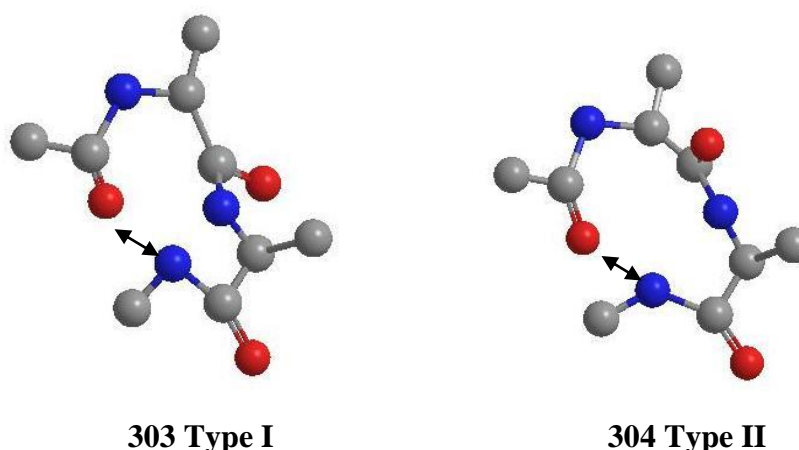


Figure 11 Diagram of type I and II turns.

Type II turn: This type of turn is similar to type I, with hydrogen bonding over three residues (**304, Figure 11**), however the backbone dihedral angles of the residue are different. The preference for amino acids also varies between the two types of turn.

Type III turn: This turn type is part of a 3.10 helix, another secondary structural element found in proteins (see later). Only a small proportion of residues are involved in 3.10 helices, and of those, nearly all the ones in helical segments contain 1-3 hydrogen bonds, with the majority less than or equal to 4 residues.

Helices: While there are a number of turns found in peptides and proteins it is the more common helix motif that is key to our investigations. Oligomeric peptides with non-polar hydrophobic side chains (e.g. polyvaline and polyleucine) are particularly prone to forming helical suprastructures.

| helix type | frequency | phi | psi | n | r(Å) | p(Å) | H-bond (CO.HN) | atoms in H-bonded loop | radius (Å) (backbone) |
|-------------|------------|--------|-------|------|------|------|----------------|------------------------|-----------------------|
| alpha | abundant | -57.8 | -47.0 | +3.6 | 1.5 | 5.5 | i, i+4 | 13 | 2.3 |
| 3.10 | infrequent | -74.0 | -4.0 | +3.0 | 2.0 | 6.0 | i, i+3 | 10 | 1.9 |
| pi | rare | -57.1 | -69.7 | +4.4 | 1.1 | 5.0 | i, i+5 | 16 | 2.8 |
| beta strand | abundant | -139.0 | 135 | +2.0 | 3.4 | 6.8 | | | |

Table 28⁶⁰ Parameters for common protein helices. (n = residues per helical turn, r = helical rise per residue, and p = helical pitch (Å/turn)).

Helices are a more stable type of secondary structure, with the most common sub type being α -helices (4-turn), however there are also 3.10 helices (3-turn) with each amino acid having a 120° turn, and π -helices (5-turn) with each amino acid having a 87° turn. α -helices are located at the core of a protein, compared with loops that are located in outer regions. Within an ideal α -helix there are 3.6 residues per complete turn, with the side chains sticking out. An example of an ideal α -helix motif is shown in **Figure 12** and **Figure 13**. The models show the overall secondary structure obtained when a long chain is formed.

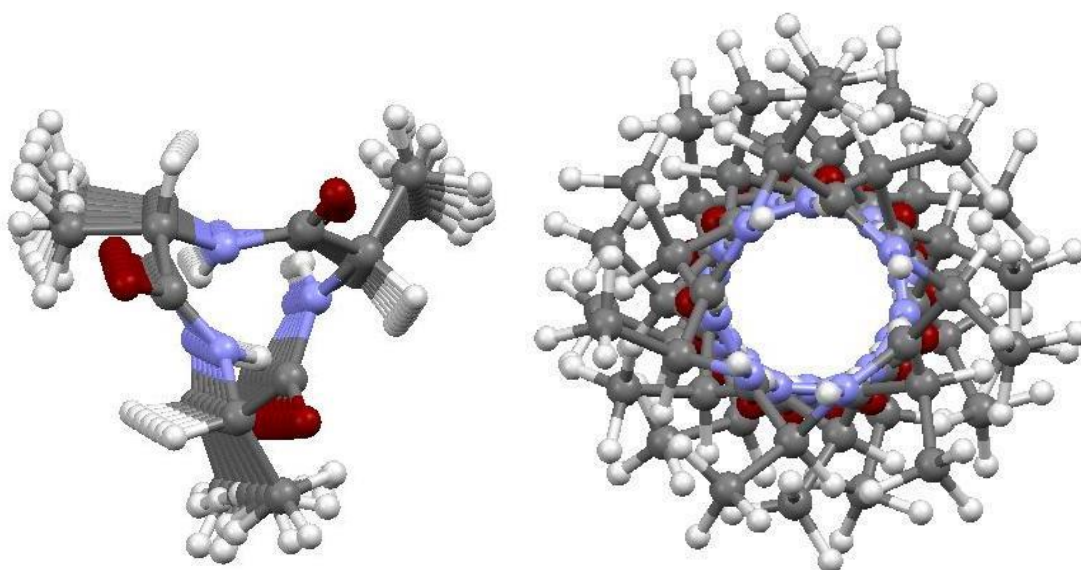


Figure 12 View down an idealised polyalanine 3.10 helix and α -helix.

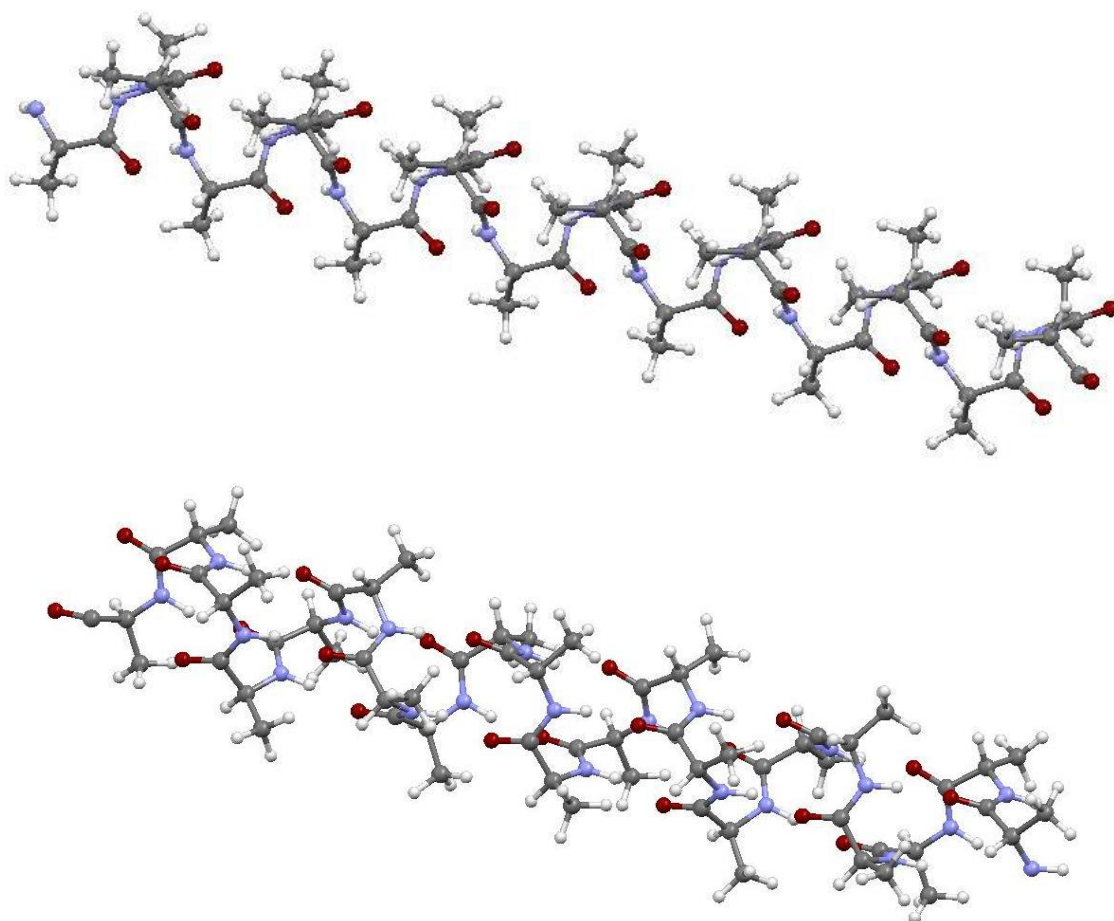


Figure 13 Sideway view of an idealised polyalanine 3.10 helix and α -helix

The structural features of turns and helices are, of course, dependant on the particular structural features of the α -amino carboxylic acids that form them. Obviously, α -amino and α -hydroxy phosphinic acids, which are constituents of our hybrid pseudopeptides have different structural features. These structural features are in turn expected to influence the turns and helical features in these pseudopeptides. For example, oligomeric α -amino carboxylic acids would contain alternating tetrahedral and trigonal carbon atoms in the chain. In contrast, oligomeric α -amino/hydroxy phosphinic acids would contain alternating tetrahedral carbon and tetrahedral phosphorus atoms. Clearly, there should be an expectation of tighter turns or turns over fewer residues.

For instance, it was decided to investigate if α -amino and α -hydroxy phosphinic acid dimers (**308**, **Figure 14**), composed of two residues with four stereocenters, would fold in a comparable manner to its tetrameric amino acid analogue (**309**, **Figure 14**) since

both molecules contain four stereocenters. Obviously, within the same parameters, hybrid pseudopeptides containing one α -amino or α -hydroxy phosphinic acid and two α -amino carboxylic acids (**305-307**, **Figure 14**) would also be likely to show folding patterns.

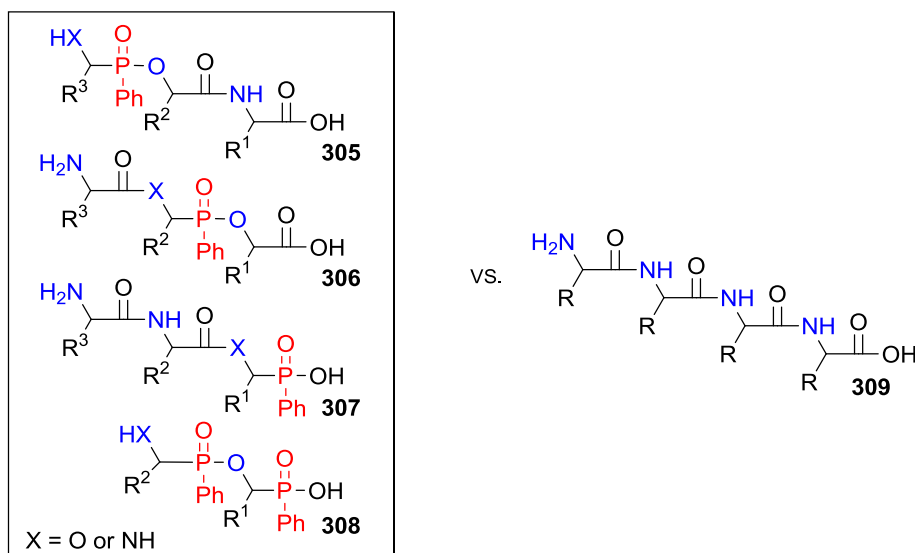


Figure 14 Proposed pseudopeptides to be synthesised compared with tetrameric amino acid analogue.

So the strategy was to synthesise, through the protocols outlined in (**Scheme 54**, **Scheme 55**) four general types of pseudopeptide with non-polar sidechains, each containing at least four stereocenters.

2.2. An overview of the synthetic methods used

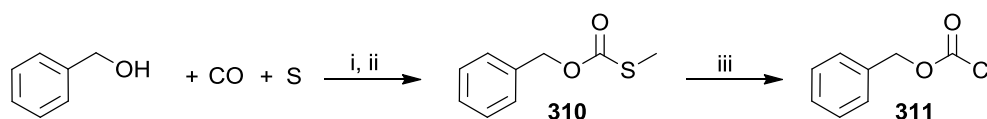
The transformations outlined in (**Scheme 54**) and (**Scheme 55**) rely on two key reactions: the Hewitt reaction and the phospho-aldol reaction. The Hewitt reaction requires a chloroformate as its starting material, whilst there are a number of methods for the phospho-aldol reaction. In this part of the thesis, consideration is given to an overview of the three key reactions that are repeatedly used in the construction of the phosphonopeptides, chloroformate synthesis, the Hewitt reaction, and the phospho-aldol reaction.

2.2.1. Chloroformate synthesis

Transformation of a hydroxyl group to the corresponding chloroformate for use in the Hewitt reaction can be carried out using different methods, including the following:

1. Carbonylation reaction⁶¹:

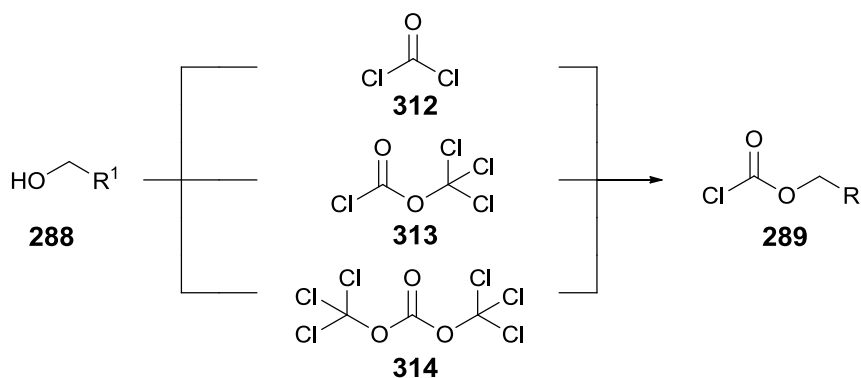
Although not a direct route to the chloroformate it is possible to carry out carbonylation of benzyl alcohols (**Scheme 56**) with carbonyl sulfide, followed by esterification with methyl iodide to give **310** and finally chlorination using sulfuryl chloride to give the chloroformate of benzyl alcohol **311**.



Scheme 56 *Reagents and conditions:* (i) DBU, THF, 80 °C, 6 h; (ii) MeI, 20 °C, 16 h; (iii) SO₂Cl₂ 0 - 20 °C, 1 h.

2. Treating the alcohol with phosgene⁶² and its synthetic equivalents:

Phosgene (**312**, **Scheme 57**) is a highly toxic, colourless, poisonous gas, used in World War I as a chemical weapon. Phosgene **312** can be used in many organic chemistry reactions, the most important being synthesis of chloroformates (**289**, **Scheme 57**), carbonates, isocyanates and acid chlorides.



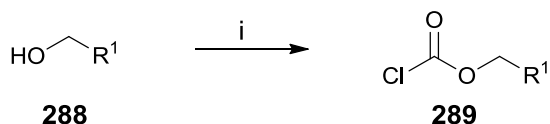
Scheme 57 Conversion of alcohol to chloroformate using phosgene and its equivalents.

Due to the toxicity of phosgene **312**, substitutes have been created: trichloromethyl chloroformate (diphosgene, **313**, **Scheme 57**) and bis(trichloromethyl) carbonate (triphosgene, **314**, **Scheme 57**).

Diphosgene⁶³ is a safer phosgene source, a liquid at room temperature that forms phosgene on heating or through catalysis.

Triphosgene⁶⁴ is a non-toxic synthetic equivalent to gaseous phosgene. It is a solid that is easy to handle and during the reaction will generate phosgene *in situ*.

It was decided to adopt the method that involves treatment of the alcohol with triphosgene in the presence of pyridine in order to form the desired chloroformate (**Scheme 58**).

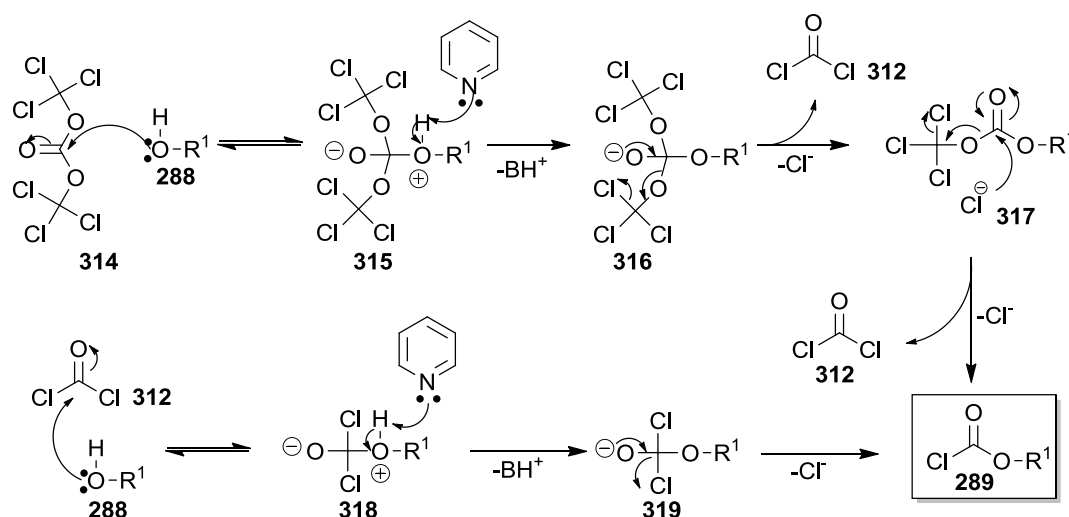


Scheme 58 *Reagents and conditions:* (i) Py, (COCl₂)₃ (**314**), diethyl ether or CH₂Cl₂, 0 °C 3 h.

In the earlier part of the work, formation of the chloroformate was carried out in diethyl ether. Pyridine was slowly added to a stirred solution of triphosgene **314** and alcohol **288** in diethyl ether, maintained at 0 °C under a nitrogen atmosphere. The reaction was typically stirred for three hours, after which the reaction was monitored by TLC and if necessary, additional reagents were added to the reaction mixture. Once the reaction was complete, the mixture was filtered, to remove pyridinium hydrochloride, and was then stripped of solvent to give the desired chloroformate **289**. Chloroformates were then analysed by spectroscopy to confirm their formation, prior to further reaction.

However, as confidence in chloroformate formation grew, it became more convenient to alter the reaction procedure. Subsequent reactions were carried out in dichloromethane, and the resulting solution, containing crude chloroformate was used directly in the next step of the Hewitt reaction.

The mechanism for formation of chloroformates from alcohols is outlined in **Scheme 59**.

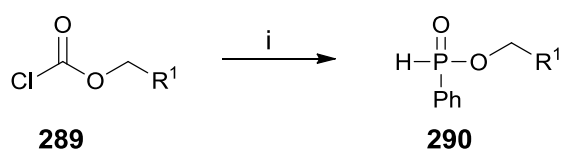


Scheme 59 Mechanism of chloroformate formation.

The first step of chloroformate formation involves the lone pair of the alcohol **288** oxygen carrying out a nucleophilic attack on the carbonyl of triphosgene **314** forming intermediate **315**. Once intermediate **315** is formed, the base removes a proton from the oxonium cation to form **316**. After loss of a proton, the tetrahedral intermediate breaks down to form a trichloromethyl carbonate **317** whilst generating a molecule of phosgene **312** and a chloride ion. The chloride ion that was released subsequently attacks the carbonyl of the intermediate formed previously, leading to formation of the desired chloroformate **289** and elimination of another molecule of phosgene **312** from the intermediate. The two molecules of phosgene **312** formed *in situ* during the reaction will then react directly with more of alcohol **288** to produce more chloroformate *via* intermediates **318** and **319**.

2.2.2. The Hewitt reaction

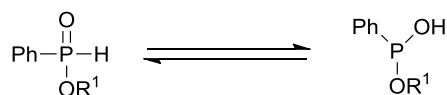
Preparation of phosphinate monoesters from phosphinic acid was a problematic reaction in organophosphorus synthetic chemistry until our group revisited an unusual reaction first reported by Hewitt.⁵⁸ Hewitt had reported that phenylphosphinic acid is transformed to its mono ethyl ester in a spontaneous reaction with a concurrent loss of CO₂ upon treatment with ethyl chloroformate and pyridine in chloroform.



R¹ = Me (73%), Et (99%) **320**, *i*Pr (88%), Allyl (80%), Benzyl (91%) **321**,
Cholestyl (93%), Decyl (85%), 2-(Benzyloxy)ethyl (85%)

Scheme 60 *Reagents and conditions:* (i) PhP(OH)₂, CH₂Cl₂, 40 °C.⁶⁵

Further investigation by our group showed that this was a general reaction and many commercially available chloroformates react with phenylphosphinic acid in the presence of pyridine, to give the corresponding phenylphosphinate monoester (**Scheme 60**). In addition, a wide range of primary and secondary alcohols can be transformed to their corresponding chloroformates and undergo the Hewitt reaction. The conversion is exceptionally clean and efficient, affording the desired phenylphosphinic monoester in high yields, usually without the need for purification. Significantly, the Hewitt reaction proceeds with retention of configuration if a chiral alcohol is used. However, carbamoyl chlorides do not undergo a similar reaction and therefore, the Hewitt reaction is not applicable to the synthesis of phosphorus-nitrogen bonds.



322 R = H, Phenylphosphinic acid

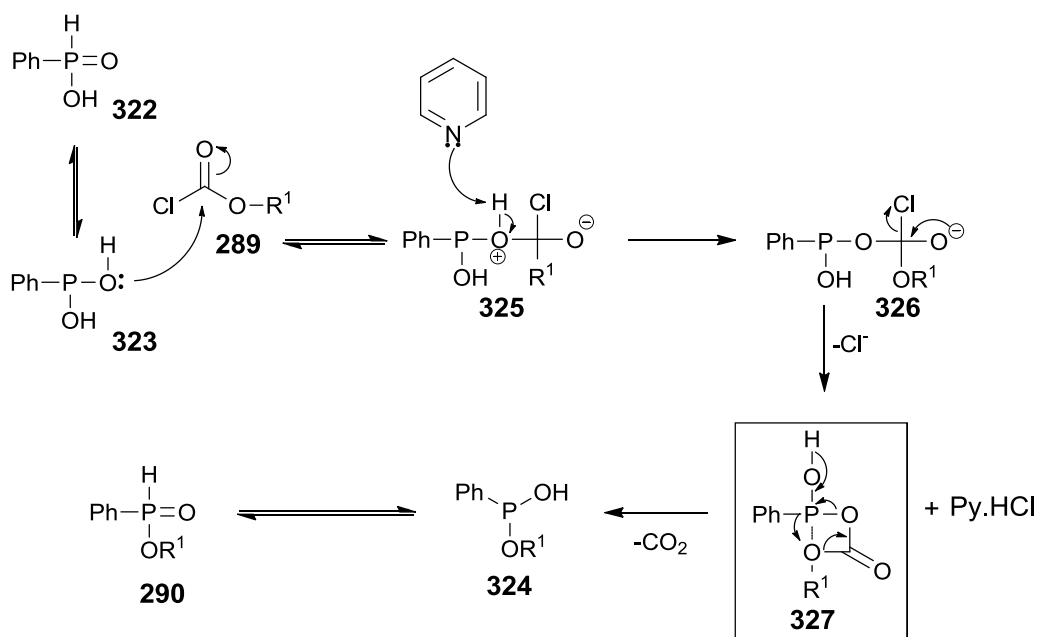
323 R = H, Phenylphosphinous acid

290 R = Alkyl etc., Phenylphosphinate ester

324 R = Alkyl etc., Phenylphosphinous mono ester

Scheme 61 Equilibrium between pentavalent and trivalent phosphonic acids and esters.

Pentavalent tetracoordinated phenylphosphinic acids and esters exist in equilibrium with their tautomeric trivalent tricoordinated phenylphosphinous acid/esters (**Scheme 61**). The consequences of this equilibrium are two fold: Firstly, although the phosphorus atom in the phenylphosphinate ester is chiral, enantiopure samples racemise very quickly through this equilibrium and are unlikely to be separable in an optically active form. Secondly, phenylphosphinous acid and its esters can demonstrate nucleophilic properties (as the phosphinous acid/ester tautomer) as well as electrophilic properties (as the phosphinate tautomer). This “dual” nature of course is also seen in keto-enol tautomers; although in this case, refers to the reactivity at the same atom (phosphorus).



Scheme 62 Proposed mechanism of the Hewitt reaction.

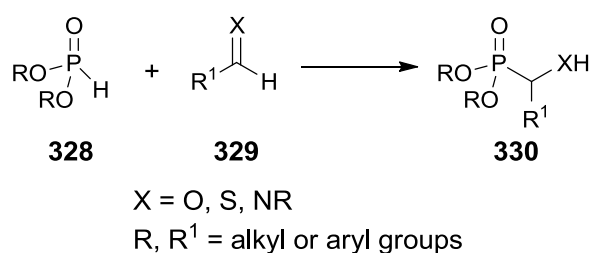
A mechanism for the Hewitt reaction is proposed (**Scheme 62**) which has been based on investigations by Afarinkia and Yu.⁶⁵

The reaction proceeds via the formation of a “mixed anhydride” intermediate **327** (**Scheme 62**). The key step in this reaction is the nucleophilic attack of phosphorus on oxygen and a concurrent loss of CO₂. This highly unusual mechanism is supported by the observation that the absolute configuration of the carbon atom in enantiopure chloroformates is retained. The phenylphosphinates cannot be separated at this point due to $\sigma^3\lambda^3$ - $\sigma^4\lambda^5$ tautomerism of H-phosphonites which makes separation impossible.

With the phenylphosphinates synthesised, reactions with aldehydes or imines can take place under phospho-aldol conditions to give the corresponding products.

2.2.3. The phospho-aldol reaction

The reaction of a phosphorous acid or ester and a carbonyl substrate (typically aldehydes or imines) (**Scheme 63**) is known as a phospho-aldol reaction. The phospho-aldol reaction is a well known reaction that has been used for the synthesis of α -functionalised phosphonates since the 1950's.⁶⁶



Scheme 63 Generalised phospho-aldol reaction.

The phospho-aldol reaction has been reported to proceed under numerous conditions,⁶⁷ with a wide range of carbonyls as well as phosphorous acid/esters.

It is also possible to control the stereochemistry at the newly created asymmetric carbon atom (for a review see: Kee *et al.*⁶⁸). This can be achieved by using a chiral aldehyde or

imine as a starting material,^{69,70,71} use of a chiral phosphorus reagent as a starting material,^{72,73,74} use of a chiral base,^{75,76} or indeed, one of many metal catalysts containing chiral ligands.^{77,78,79,80,81,82}

During the present work, KF was used as a means of activation of the carbonyl of the aldehyde during the phospho-aldol reaction for some reactions. This solvent free method used with liquid aldehydes, gives clean high yielding products, compared with thermally mediated phospho-aldol reactions.

The most common types of the reaction are those with pentavalent tetracoordinated phosphinate mono or diesters (also known as hydrogen phosphites). However trivalent tricoordinated phosphorous triesters (also known as triphosphites) also undergo an efficient reaction (Abramov reaction).^{66,83,84} There are examples of the Kabachnik-Fields reaction; a three component carbonyl, amine and hydrophosphoryl coupling using catalysts to give α -amino phosphonates,^{85,86} and enantiomerically pure α -amino phosphonates.^{87,88}

Although the outcomes of the two types of phospho-aldol reaction are similar, they proceed by different pathways. As was discussed earlier (**Scheme 60**), pentavalent tetracoordinated phosphonite monoacids are not nucleophilic at all. They are in fact more susceptible to be electrophiles. However, because of the equilibrium between the pentavalent tetracoordinated phosphorous acid and its trivalent tricoordinated tautomer, there is always a small concentration of the latter in any reaction mixture. In this nucleophilic form, phosphorous species react with electrophilic carbonyls. The rate of the addition can be enhanced by addition of a base, which may drive the phosphorous tautomerism towards the anionic species, or a Lewis acid which activates the carbonyl function.

Trivalent tricoordinated phosphorous trialkyl esters are already in a nucleophilic form and readily attack carbonyls, however the addition is reversible. These reactions can be promoted thermally or by addition of protic and Lewis acids, which activate the carbonyl function and facilitate the loss of an alkyl group.

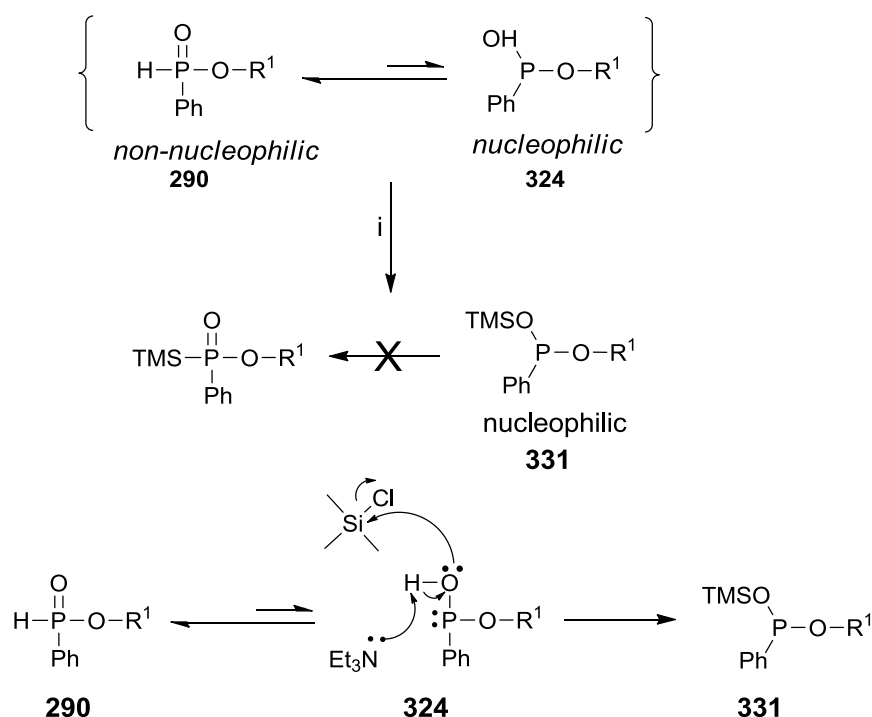
In between these two types are the phosphorous silyl esters. Like their trialkyl ester analogues, phosphorous silyl esters are already in a nucleophilic form and react readily with carbonyl functions. However, unlike their trialkyl ester analogues, the adduct breaks down very easily, driven by concurrent migration of the silyl group to the oxygen of the carbonyl (or the nitrogen of imine). Earlier computational investigations have suggested that the addition may be a concerted process, but even so, the overall reaction is facile.

2.2.3.1. The silyl mediated phospho-aldol reaction

One of the most significant advances in the phospho-aldol reaction in recent years has been the development of the “*in situ*” method for the preparation of silyl esters of phosphorous acids.⁸⁹ The *in situ* method circumvents problems with handling of the hydrolytically sensitive phosphorous silyl esters. During the *in situ* method, a solution of phenylphosphinate is treated sequentially with dry triethylamine and TMSCl, which generates *in situ* the phenylphosphinous tautomer. This will in turn react with the aldehyde or imine under very mild conditions.

The mechanisms through which the reactions take place are outlined in **Scheme 64**. As outlined in **Scheme 61**, pentavalent tetracoordinated phenylphosphinic acids and esters **290** exist in equilibrium with their tautomeric, trivalent tricoordinated phenylphosphinous acid/esters **324**. Whilst the trivalent tricoordinated tautomer is nucleophilic, the equilibrium is overwhelmingly in favour of the non-nucleophilic pentavalent tetracoordinated tautomer. The lack of reactivity of phosphorous acids

towards aldehydes and imines can be attributed to the status of this equilibrium. Treatment with dry triethylamine and TMSCl converts the phosphorous acid to its silyl ester. Due to the strong Si-O bond, the phosphorous silyl esters are locked in the nucleophilic trivalent tricoordinated form **324**. At the same time as the **290** tautomer is converted to **324**, the equilibrium drives forward, eventually resulting in complete conversion of the equilibrium mixture to **331**.

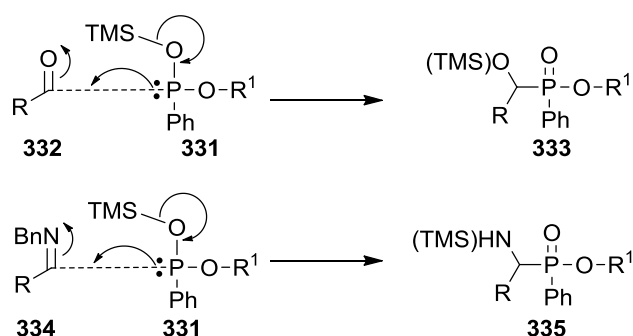


Scheme 64 Reagents and conditions: (i) Et₃N, TMSCl.

Once the molecule is locked in the nucleophilic form addition of the aldehyde or imine can take place according to the mechanism shown in **Scheme 65**.

Silyl-mediated phospho-aldol reactions initially afford the O-silylated α -hydroxy or N-silylated α -amino phenylphosphinates. As the N-Si bond is labile and easily susceptible to hydrolysis, they were not usually observed. Since the O-Si bond is more robust, after aqueous workup or during chromatographic purification on silica gel, partial cleavage of the silyl ethers is observed. In order to ensure that this did not cause a problem with purification, crude reaction mixtures were treated with tetrabutylammonium fluoride

(TBAF) to remove the silyl ether. Silicon carbon bonds are polar, leaving the TMS protecting group open to nucleophilic attack. A source of fluoride acts as a strongly electronegative nucleophile that can form a strong bond to silicon. Silicon can form a strong bond with fluorine with a value of $522.7 \text{ kJ mol}^{-1}$ that facilitates the deprotection. TBAF is supplied as a 1.0M solution in THF; therefore the deprotection is carried out in a THF/diethyl ether mixture.



Scheme 65 Phospha-aldol reaction of phosphonite and aldehyde or imine to form phosphinate monomer.

2.3. Synthesis of α -hydroxy and α -amino phenylphosphinate monomers

In line with the general synthetic strategy outlined in **Scheme 54**, the first task was to prepare α -hydroxy and α -amino phenylphosphinate monomers (**Figure 15**).

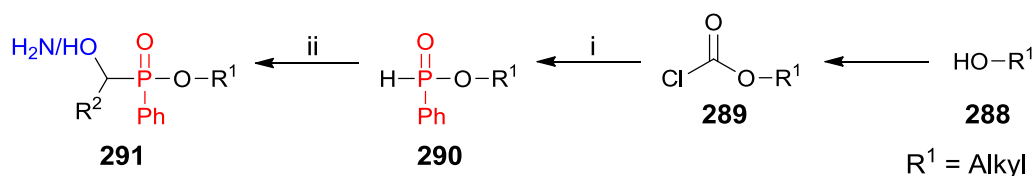
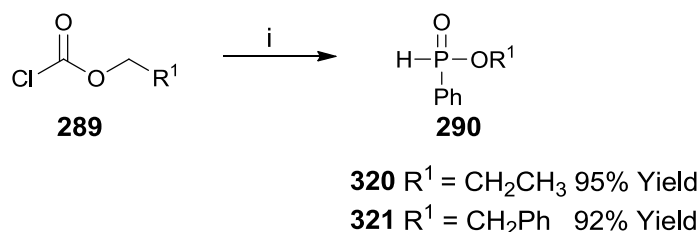


Figure 15 (i) Hewitt reaction; (ii) phospha-aldol reaction (332/334).

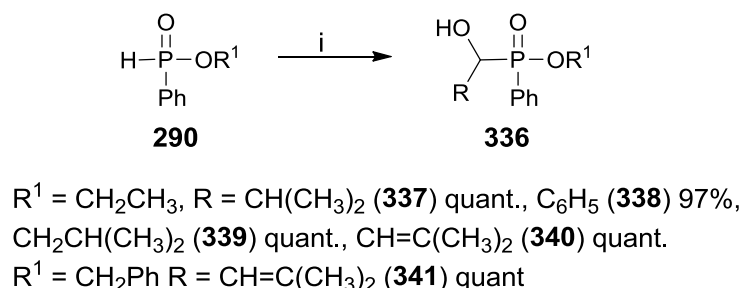
Ethyl and benzyl chloroformates were reacted with phenylphosphinic acid under Hewitt reaction conditions. The reactions proceed cleanly and efficiently to afford 95% and

92% yield of **320** and **321** respectively (**Scheme 66**). Both compounds were sufficiently pure after work up to be used directly in the next step of the synthesis.



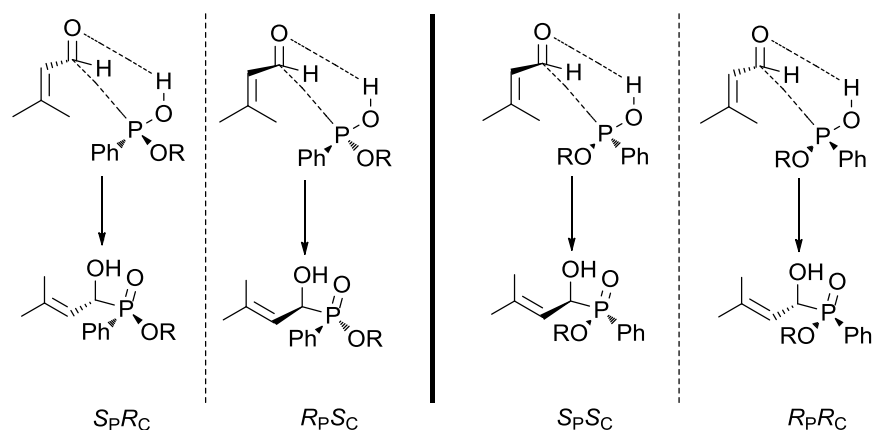
Scheme 66 *Reagents and conditions:* (i) Py, PhP(OH)₂, CH₂Cl₂, 40 °C, 30 min.

As mentioned in **Section 2.2.3** alkyl phenylphosphinates react with aldehydes under a variety of conditions, including in the presence of KF, to afford the corresponding α -hydroxy phenylphosphinates. Indeed, both benzyl and ethyl phenylphosphate react with a number of aldehydes efficiently and cleanly (**Scheme 67**).



Scheme 67 *Reagents and conditions:* (i) RCHO, KF, overnight, then CH₂Cl₂, stirring 30 min.

During the phospho-aldol reaction, two new stereogenic centers are created, one at the phosphorus and the other at the hydroxyl carbon adjacent to it. Therefore, we can expect a mixture of two diastereomers. The reactions afford equal mixtures of diastereomers (**Table 29**), except in the reaction of 3-methylbut-2-enal with ethyl phenylphosphinate, where there is a modest selectivity, which can be detected in the ³¹P and ¹H NMR of the crude product, which shows a 3:2 ratio in favour of the (*S*_P,*R*_C/*R*_P,*S*_C) diastereomer. It is believed that the (*S*_P,*R*_C) and (*R*_P,*S*_C) enantiomeric pair are preferentially formed over the (*S*_P,*S*_C) and (*R*_P,*R*_C) enantiomeric pair, due to optimal π -stacking, when the phenyl ring is orientated above the alkene (**Scheme 68**).



Scheme 68 Orientations for reaction of phosphonite and aldehyde during phospho-aldol reaction.

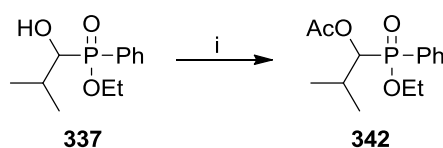
From the reaction of benzyl phenylphosphinate two diastereomeric products (each as a pair of enantiomers) were obtained. However, it was not possible to isolate either of the diastereomers in pure form by fractional crystallisation. In contrast, from the reaction of ethyl phenylphosphinate, two diastereomers were obtained, one of which was an oil and the other one crystalline. Therefore it was possible to conveniently carry out a separation of the diastereomers by crystallisation. In the first instance, the solid diastereomer was separated from the crude product mixture and washed using diethyl ether. Recrystallisation using ethyl acetate (EtOAc) then gave colourless crystals of a single diastereomer as confirmed by ^{31}P and ^1H NMR. The absolute configuration of the two enantiomers in the crystalline diastereomer was confirmed by X-ray crystallography as $(S_P, R_C/R_P, S_C)^{90}$.

| No. | R^1 | R | Yield (%) | State | Separable |
|-----|-------------------------------------|---------------------------------------|-----------|---------------|-----------|
| 337 | CH_2CH_3 | $\text{CH}(\text{CH}_3)_2$ | Quant. | Oil Mix | No |
| 338 | CH_2CH_3 | C_6H_5 | Quant. | Crystal Mix | No |
| 339 | CH_2CH_3 | $\text{CH}_2\text{CH}(\text{CH}_3)_2$ | Quant. | Oil Mix | No |
| 340 | CH_2CH_3 | $\text{CH}=\text{C}(\text{CH}_3)_2$ | Quant. | Oil / Crystal | Yes |
| 341 | $\text{CH}_2(\text{C}_6\text{H}_5)$ | $\text{CH}=\text{C}(\text{CH}_3)_2$ | 97 | Crystal Mix | No |

Table 29 α -hydroxy monomer compounds synthesised.

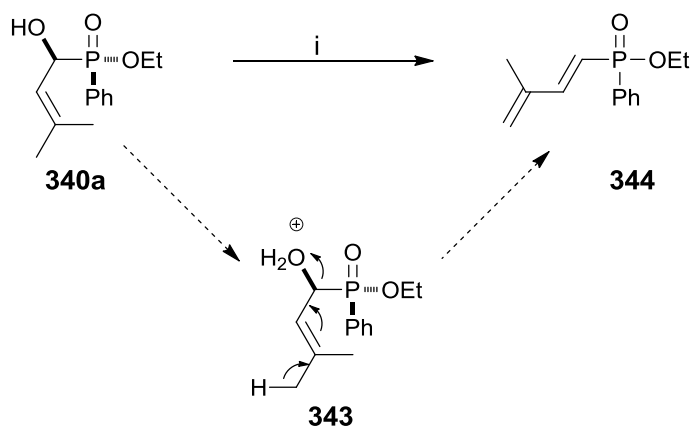
Although it was possible to obtain one of the two diastereomers in pure form, it was not possible to get a sufficiently large quantity of the other diastereomer. Even very slow, careful crystallisation always left some of the ($S_P, R_C/R_P, S_C$) diastereomer in the supernatant solution, which meant a diastereomerically mixed residue. Not only was crystallisation not suitable for the separation of diastereomers, but chromatography also proved to be ineffective. In most cases the two diastereomers had very similar retention factors (R_f) on silica gel and were either wholly inseparable by column chromatography or could only afford a very small quantity of the pure diastereomer.

Brief investigations were carried out to see if the diastereomers could be separated chromatographically after derivatisation. Various attempts at O-alkylating the leucine analogue **340** were carried out, but were unsuccessful despite many variations in reaction conditions.



Scheme 69 Reagents and conditions: (i) Ac_2O , cat. H_2SO_4 , CH_2Cl_2 .

Acetylation of valine analogue **337** was successful giving **342** (**Scheme 69**) although the diastereomers were still inseparable.



Scheme 70 Reagents and conditions: (i) Ac_2O , cat. H_2SO_4 , CH_2Cl_2 .

Interestingly though, when (1-hydroxy-3-methylbut-2-enyl) phenylphosphinic acid ethyl ester **340a** was treated under similar reaction conditions, the expected product was not obtained (**Scheme 70**). The reaction was monitored by TLC and the starting material was completely consumed, however after work-up and NMR analysis it was clear that the desired product had not been formed.

The structure was assigned based on NMR and mass spectroscopy as **344**. ^{31}P NMR showed a signal at 31.6 ppm, ^1H NMR showed three signals for the two sets of olefinic protons 5.23, 5.84, 7.09 ppm, ^{13}C NMR showed signals at 120, 124, 150 ppm. While these characteristics signals do not confirm the compound structure, a similar compound was previously synthesised and characterised by our group showing similar spectral properties (**Figure 16**).

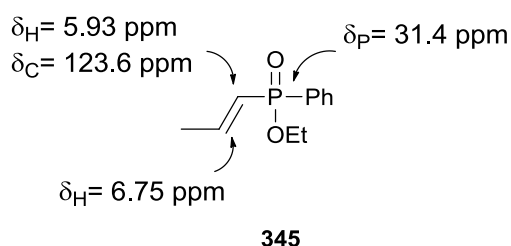
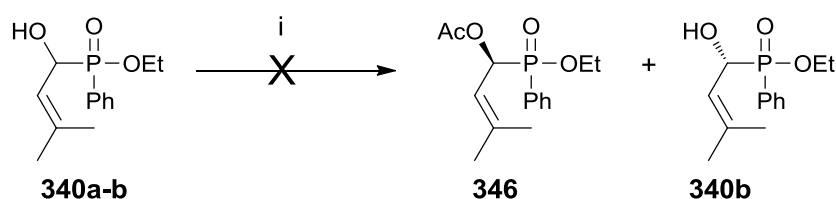


Figure 16 NMR characteristics of compound **345**.

The assignment for **344** is consistent with the corresponding values observed in compound **345** which was previously synthesised in our group.⁹¹

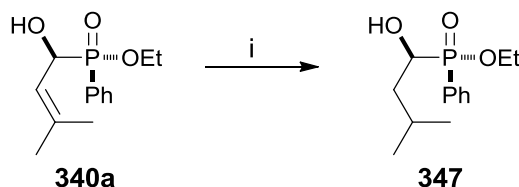
We also attempted the preparation of a single enantiomer of diastereomerically pure **340a** or **b** through an enzyme mediated acylation reaction (**Scheme 71**).



Scheme 71 Reagents and conditions: (i) vinyl acetate, Cal B, mol. sieves, diisopropyl ether, 60 °C, 2 d.

Literature procedures using enzymes to selectively protect the hydroxyl group of compounds were carried out.^{92,93} *Candida antarctica* lipase B (Cal B / Novozyme 435) and vinyl acetate in isopropyl ether were heated to 60 °C in the presence of molecular sieves to selectively acetylate one isomer of **340a-b** as in the literature but after two days no reaction had taken place, the starting material was recovered.

At this point it was decided to continue the synthetic strategies outlined in **Scheme 54** and **Scheme 55** using the crystalline diastereomer as a monomer unit for chain extension.



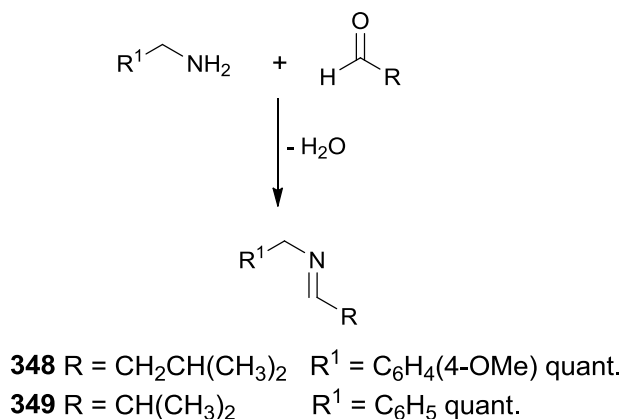
Scheme 72 Reagents and conditions: (i) Pd/C (10% wt), H₂, EtOAc, 100%.

The next step of the synthesis involves hydrogenation of the double bond (**Scheme 72**). Ethyl 1-hydroxy-3-methylbut-2-enyl(phenyl)phosphinate **340a** underwent hydrogenation at standard pressure and temperature using Pd/C as the catalyst with EtOAc as solvent. The hydrogenated product **347** serves as the phosphono leucine monomer unit, which is analogous to leucine, but contains two stereocenters per residue unlike the single stereocenter in each leucine residue. It should be noted that **347** could not be prepared in a diastereomerically pure form by treatment of **320** with isovaleraldehyde as shown in **Scheme 67**. The reaction affords a mixture of diastereomers that are not separable.

As well as synthesising alcohol monomers concurrent synthesis of a number of amino monomers was carried out. This allowed for variation in the pseudopeptides formed, due to flexibility in the structure by inclusion of the amine functionality that can be

derivatised, whilst still providing a hydrogen donor for use in secondary structural properties.

To this end the synthesis of two different types of amine monomers were synthesised using imines prepared according to **Scheme 73**.



Scheme 73 Reagents and conditions: (i) toluene, 120 °C, overnight.

Formation of the imines were carried out by refluxing isovaleraldehyde and the corresponding amine in toluene overnight, using Dean-Stark apparatus to collect the water generated during the reaction (**Scheme 73**). Imines **348** and **349** were not purified, but reacted directly with phosphite **320** under phospho-aldol conditions to give amine monomers **350** and **351** (**Table 30**).

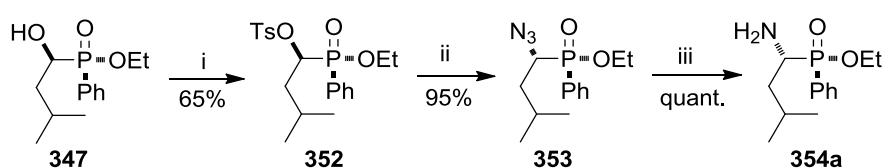
| $ \begin{array}{c} \text{R}^1\text{HN} \\ \\ \text{C} - \text{P}(=\text{O})(\text{Ph})(\text{OEt}) \\ \\ \text{R} \end{array} $ | | | | | |
|---|--|---|---------------|-----------|-----------|
| No. | R ¹ | R | State | Yield (%) | Separable |
| 350 | CH ₂ (C ₆ H ₄ - <i>p</i> OMe) | CH ₂ CH(CH ₃) ₂ | Oil / Oil | Quant. | No |
| 351 | CH ₂ (C ₆ H ₅) | CH(CH ₃) ₂ | Oil / Crystal | 95 | Yes |

Table 30 Amine monomers synthesised.

As shown in **Table 30**, amine monomers were successfully synthesised, however isolation of all of the diastereomers proved difficult apart from with valine analogue **351**. As the initial work focused on synthesis using leucine analogues the use of the

valine analogue was put on hold until further work with the leucine analogue was carried out.

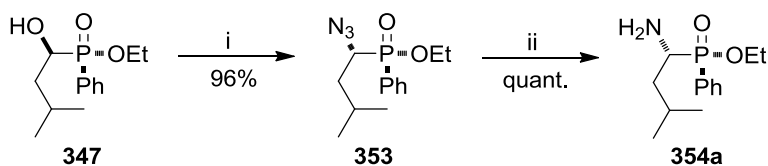
In view of the problem with the isolation of pure diastereomers from the aldol reaction with benzyl imines, an alternative strategy was envisaged for the formation of the α -amino phenylphosphinate monomer. This was the transformation of the compound **347**, either directly or via its tosyl or mesyl derivative, to its azide derivative, followed by reduction of the azide function to amine.



Scheme 74 Reagents and conditions: (i) TsCl, Py, 0 °C to rt; (ii) NaN₃, DMF, 60 °C; (iii) Pd/C 5%, H₂, EtOAc.

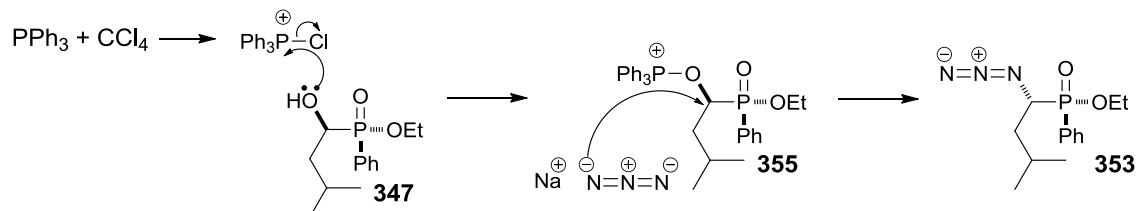
Alcohol **347** was transformed into the corresponding *p*-toluenesulfonyl (tosyl) derivative **352** by reacting with tosyl chloride in pyridine (**Scheme 74**). Introduction of a tosyl group produces a good leaving group that can be reacted with nucleophiles and easily be displaced. Compound **352** was reacted with sodium azide in DMF to give azide **353**. The last step involved reduction of the azide to give amine compound **354a** as a yellow oil. This reduction was carried out with hydrogen using Pd/C as the catalyst and EtOAc as the solvent.

Whilst the classical azide formation method was suitable, a shorter method⁹⁴ was adopted and is described in **Scheme 75**:



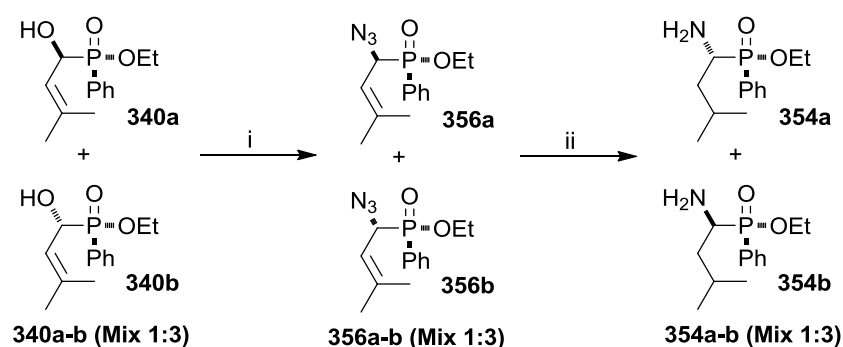
Scheme 75 Reagents and conditions: (i) PPh₃, NaN₃, CCl₄, 60 °C; (ii) Pd/C 5%, H₂, EtOAc.

Compound **347** was reacted with triphenylphosphine and sodium azide in carbon tetrachloride, to give after column chromatography compound **353** as previously discussed (**Scheme 74**).



Scheme 76 Mechanism for transformation of alcohol to azide.

The mechanism for the conversion of hydroxyl **347** to azide **353** goes *via* an S_N2 mechanism shown in **Scheme 76**. The first part of the reaction involves the formation of the chlorotriphenylphosphonium ion, which occurs when triphenylphosphine and carbon tetrachloride react with each other. The lone pair of the hydroxyl group attacks the phosphorus atom of the chlorotriphenylphosphonium ion forming intermediate **355**, which is attacked by sodium azide to form the corresponding azide product with inversion of configuration at the stereocenter. Therefore, the reaction presumably afforded compound **353** with (R_P, R_C) relative configuration.



Scheme 77 Reagents and conditions: (i) PPh_3 , NaN_3 , CCl_4 , 60 °C; (ii) Pd/C 5%, H_2 , $EtOAc$.

In an attempt to investigate if the amine with R_P, S_C relative configuration can be obtained, the same reaction was carried out using the sample of alcohol **340a-b** enriched with the non-crystalline diastereomer (R_P, R_C relative configuration) (**Scheme 77**).

However, both azides **356a-b** and amines **354a-b** were obtained as an inseparable diastereomeric mixture.

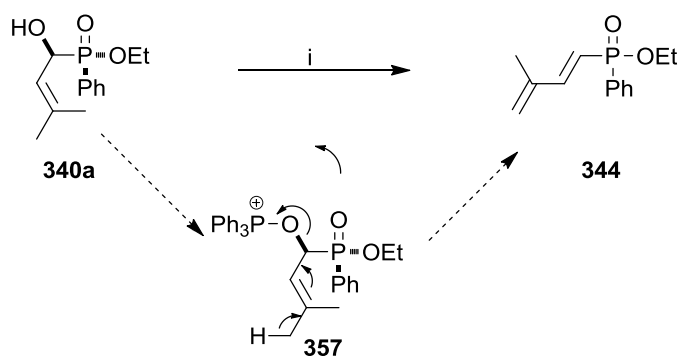
2.3.1. Unexpected reactions when forming the azide

Further probing of the reaction between triphenylphosphine, sodium azide and carbon tetrachloride for formation of the azide with compound **340a** was carried out in an attempt to save one step by reducing the double bond and the azide in one reaction.

Triphenylphosphine and sodium azide were added in a large excess to **340a** in carbon tetrachloride and heated to 80 °C for 3 hours, varying the original conditions significantly. It had been anticipated that the reaction could be accelerated and also reduce the synthesis by one step.

After heating for three hours the starting material had been consumed and a product with similar R_f to azide **353** had been formed. However after purification of the compound the ^1H NMR was not in agreement with the azide product but instead a new product had been formed.

The compound formed during this reaction is identical to compound **344** formed when reacting the alcohol with acetic anhydride in the presence of acid (Scheme 70).



Scheme 78 Reagents and conditions: (i) PPh_3 , NaN_3 , CCl_4 , 80 °C, 3 h.

After formation of product **344** through the reaction described in **Scheme 78** the next step was to test the reaction with a mixture of diastereomers **340a-b** (Oil/Crystal 3:1) to see the outcome of the reaction. The reaction removes one stereocenter therefore it was expected reacting the oil diastereomer would form product **344** as with the reaction of crystal diastereomer **340a** (**Scheme 70**).

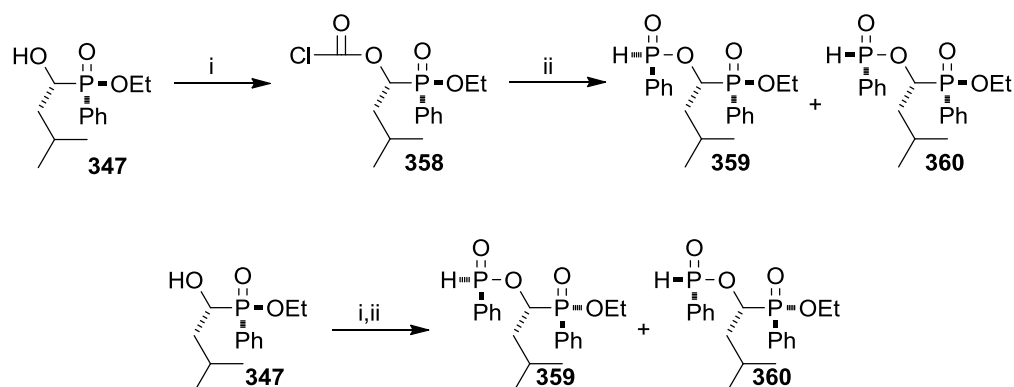
2.4. Synthesis of α -hydroxy phenylphosphinate dimers

Once a reliable method for the large scale preparation of a single diastereomeric α -hydroxy phenylphosphinic analogue of leucine was in place, it was decided to first investigate the preparation of dimeric phenylphosphinates. This required development of an iterative process in which an α -hydroxy phenylphosphinic moiety acts as the starter alcohol unit for the subsequent Hewitt and phospho-aldol reactions.

2.4.1. Forming the chloroformate of the monomer & the Hewitt reaction

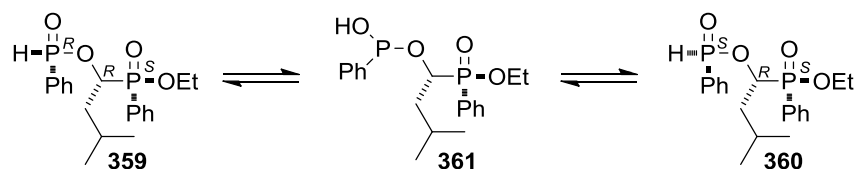
Formation of dimers requires conversion of the hydroxyl group of compound **347** to the chloroformate **358** (**Scheme 79**) as previously shown in **Scheme 58** followed by Hewitt reaction conditions as detailed in **Scheme 60** to give the corresponding phenylphosphinate **359-360** (**Scheme 79**).

Initially, synthesis of phenylphosphinate **359-360** was carried out in two steps (**Scheme 79**), with characterisation of intermediate chloroformate **358** to ensure its efficient and successful formation. However, once confidence in the reliability of the reaction increased, further syntheses of phosphite **359-360** were carried out in a modified “one pot” synthesis (**Scheme 79**). The mechanisms for formation are the same as the general mechanisms outlined in **Scheme 59** and **Scheme 62**.



Scheme 79 Reagents and conditions: (i) Py, (COCl₂)₃, CH₂Cl₂, 0 °C, 3 h; (ii) PhP(OH)₂, CH₂Cl₂, 40 °C, 1 h.

In the “one-pot” method, the complete formation of the intermediate chloroformate was monitored by TLC and confirmed by ³¹P NMR. In the event of the reaction not having been completed, additional reagents were added and the monitoring of the reaction was continued. Once the chloroformate had been completely formed, the reagents for the Hewitt reaction were added carefully and the reaction was heated at reflux for one hour whilst being checked periodically by TLC.



Scheme 80 Equilibrium between H-phosphonite tautomers.

The product of the reaction is a diastereomeric mixture of (*S_P*,*R_C*,*R_P*) and (*S_P*,*R_C*,*S_P*) relative configurations. Separation of these diastereomers proved to be impractical as the two are interconvertible due to σ³λ³-σ⁴λ⁵ tautomerism of H-phosphonites (**Scheme 80**). Thus the two diastereomers **359-360** are in equilibrium with the phenylphosphinous mono ester tautomer **361** which nevertheless exists in only minute amounts. In the ³¹P NMR two pairs (i.e. four signals) were observed, each as a doublet due to P-P coupling (**Figure 17**). This observation is consistent with two diastereomers being present in equal ratios. Each diastereomer has two doublets, one signal for each phosphorus atom that is in turn split by the other phosphorus atom.

The addition of an aldehyde to phosphonite **359-360** creates two additional stereocenters in the products, leading to the formation of four diastereomers. Since each of the diastereomers contains two phosphorus atoms in different magnetic environments, they each appear with a different signal and couple to each other. Therefore, in the absence of any selectivity, a total of 16 peaks in the ^{31}P NMR would be repeated.

The silylated phosphorus esters were formed by addition of TMSCl to a solution of triethylamine and compound **359-360** in dichloromethane at 0 °C. After 15 minutes the aldehydes were added and the mixture was stirred at room temperature overnight. The reaction was monitored by withdrawing a small sample which was then evaporated, and analysed by NMR.

After confirmation that the reaction was complete, the mixture was concentrated and subjected to an aqueous work up and additional analysis by NMR. Typically at this stage, the ^{31}P NMR showed a change from 16 peaks to 32 peaks, 16 peaks corresponding to the four silylated products **362-364**, with an additional 16 peaks corresponding to the hydrolysed alcohol products **365-367**. As the silyl group had been partially removed during work up it was deemed necessary to carry out a complete removal of the silyl group using TBAF so that the separation of products would be simplified.

After treatment with TBAF, column chromatography was carried out to separate out the four diastereomers. The clean separation of all four of the diastereomers by column chromatography was not always possible. However, in most cases, it was possible to obtain sufficient quantities of pure diastereomers for further analysis.

2.4.2.1. Dimer (365) formed using benzaldehyde

The first dimer to be synthesised was **365**, with the relevant chemical properties described in **Table 31**.

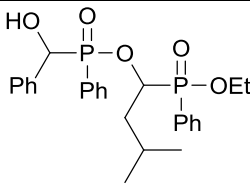
|  <p>Diastereomeric ratio: 7:3:1:1</p> | | | | | |
|--|----------------|-----------------|----------|-------|-----------------------|
| No. | R _f | Stereochemistry | State | X-ray | ³¹ P Peaks |
| 365a | 0.62 | <i>SRRR</i> | Crystal | Yes | 40.65, 43.15 |
| 365b | 0.59 | <i>SRRS</i> | Crystal | Yes* | 39.8, 40.65 |
| 365c | 0.30 | <i>SRSS</i> | Oil Mix. | No | 37.84, 40.82, |
| 365d | 0.30 | <i>SRSR</i> | | | 38.51, 40.38 |

Table 31 Chemical properties of dimer **365** (*crystals previously isolated and characterised).

2.4.2.2. Dimer (366) formed using isovaleraldehyde

After the reaction with benzaldehyde the next aldehyde chosen was isovaleraldehyde. The reaction was carried out as shown in **Scheme 81** using isovaleraldehyde. The NMR spectra showed the same patterns as seen when reacting with benzaldehyde. The chemical properties for dimer **366** are described in **Table 32**.

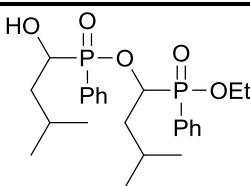
|  <p>Diastereomeric ratio: 2.5:2:1:1</p> | | | | | |
|--|----------------|-----------------|---------|-------|-----------------------|
| No. | R _f | Stereochemistry | State | X-ray | ³¹ P Peaks |
| 366a | 0.7 | <i>SRRR</i> | Oil | No | 40.25, 46.05 |
| 366b | 0.4 | <i>SRRS</i> | Crystal | Yes | 41.0, 42.1 |
| 366c | 0.25 | <i>SRSS</i> | Solid | No | 38.8, 41.8 |
| 366d | 0.24 | <i>SRSR</i> | Crystal | Yes | 38.2, 41.0 |

Table 32 Chemical properties of dimer **366**.

2.4.2.3. Dimer (367) formed using isobutyraldehyde

The addition of isobutyraldehyde was carried out as shown in the previous aldehyde additions to give dimer **367**, with the chemical properties described in **Table 33**.

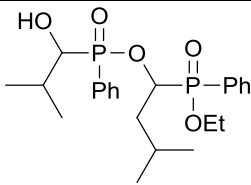
|  <p>Diastereomeric ratio: 2.4:2.3:1:1</p> | | | | | |
|--|----------------|-----------------|---------|-------|----------------------------|
| No. | R _f | Stereochemistry | State | X-ray | ³¹ P Peaks |
| 367a | 0.6 | <i>SRRR</i> | Crystal | Yes | 41.26, 45.23 |
| 367b | 0.55 | <i>SRRS</i> | Crystal | Yes | 41.57, 42.55 |
| 367c | 0.35 | <i>SRSS</i> | Crystal | Yes* | 39.06, 41.81, 39.53, 41.81 |
| 367d | 0.34 | <i>SRSR</i> | Crystal | Yes* | |

Table 33 Chemical properties of dimer **367** (*crystals previously isolated and characterised).

Detailed analysis of the solution structures of diastereomers in the same series were carried out through NMR spectroscopy, including $^1\text{H}\{^{31}\text{P}\}$ spectroscopy, NOESY (Nuclear Overhauser Enhancement Spectroscopy), and HOESY (Heteronuclear Overhauser Enhancement Spectroscopy) between phosphorus and hydrogen nuclei. During the analysis of the ^1H NMR spectra it was clear that the first two diastereomers in the isovaleraldehyde series had a different conformation to the latter two. On comparison of the ^{31}P -coupled and ^{31}P -decoupled ^1H spectra ($^1\text{H}\{^{31}\text{P}\}$) it could be seen that in the first two diastereomers the hydroxyl hydrogen was coupled to a hydrogen nucleus (H_β) and both phosphorus nuclei. HOESY between the phosphorus and hydrogen nuclei (**Figure 18**) showed that the hydroxyl hydrogen is coupled slightly more strongly to the phosphorus nucleus bonded further away from it (P_α) than to the phosphorus nucleus bonded closest to it (P_β). The same studies were carried out on the other two diastereomers in the series and the results showed that the hydroxyl hydrogen

is coupled to a hydrogen nucleus (H_β) and the phosphorus nucleus bonded closest to it only (P_β). Based on the observations it could be suggested that the coupling between the hydroxyl and P_α in the first two diastereomers cannot be through-bond but must be through-space, the most likely way this could take place would be through intramolecular hydrogen bonding. To confirm the coupling did not arise due to intermolecular hydrogen bonding successive ten-fold dilutions were carried out that did not affect the spectra, thus proving the theory. The same observations were shown when reacting with the other aldehydes in the series.

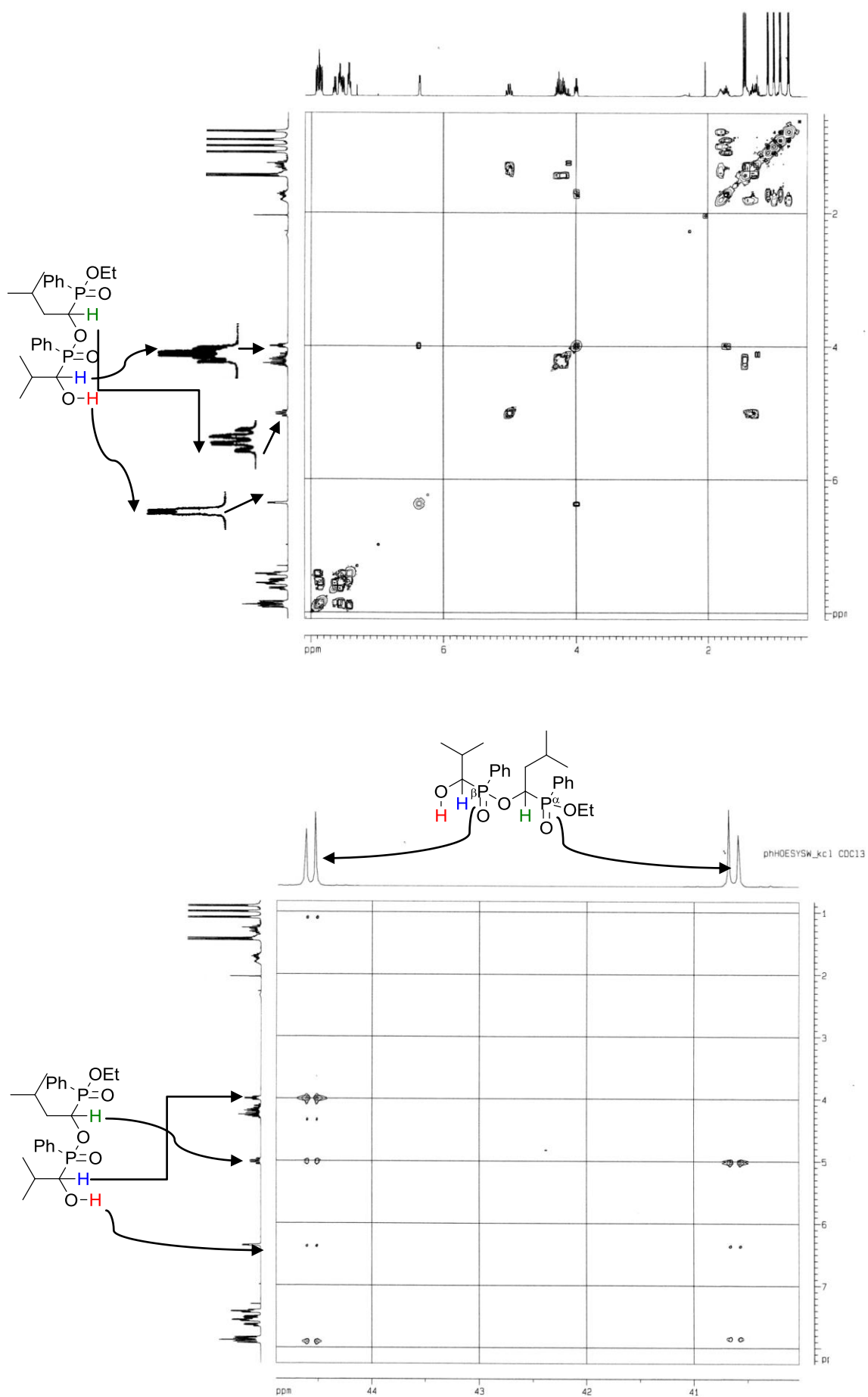


Figure 18 2D NMR spectra of compound **367a**.

2.4.3. Computational Studies of α -hydroxy phenylphosphinate dimers¹

To examine the propensity of compounds **366a-d** to form intramolecular hydrogen bonds from a theoretical perspective, a randomized (Monte Carlo) search of their conformational preferences was carried out.⁹⁵ The results of these computations are shown in **Table 34**. Starting from four different starting conformations, 1296 random conformations were analysed. In each case, the lowest energy conformations were visually inspected. In two cases, diastereomers (S_P, R_C, R_P, R_C) and (S_P, R_C, R_P, S_C), the lowest energy conformations as well as a number of other low energy conformations were found to contain an intramolecular hydrogen bond between the hydrogen atom of the terminal hydroxyl group and the oxo atom (P=O) furthest away from it (**Figure 19**).

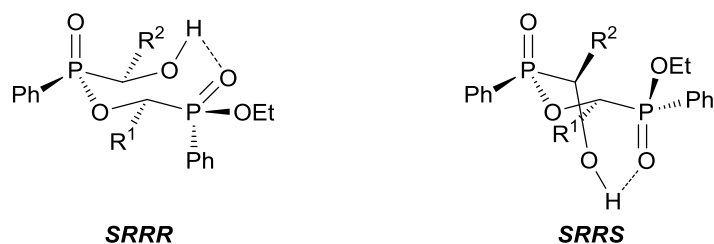


Figure 19 H-bonding interaction in two diastereomers.

In both cases, the conformations without this hydrogen bond were, as expected, higher in energy by at least 4 Kcal/mol. In the other two cases, diastereomers (S_P, R_C, S_P, S_C) and (S_P, R_C, S_P, R_C), the lowest energy conformations as well as other low energy conformations were found to contain no intramolecular hydrogen bond. In fact, no hydrogen bonded conformations were found within 10 Kcal/mol of the lowest energy conformation.

¹ This work was carried out by Dr. K. Afarinkia using Gaussian 03 and is presented here in order to provide a more comprehensive analysis.

| Relative Configuration | Lowest energy in H-bonded conformations (Kcal/mol) | Lowest energy in non H-bonded conformations (Kcal/mol) | Number of intramolecularly H-bonded conformations |
|--|--|--|---|
| (<i>S_P</i> , <i>R_C</i> , <i>R_P</i> , <i>R_C</i>) | 124.134 | 129.155 | 22 |
| (<i>S_P</i> , <i>R_C</i> , <i>R_P</i> , <i>S_C</i>) 366b | 124.141 | 128.206 | 13 |
| (<i>S_P</i> , <i>R_C</i> , <i>S_P</i> , <i>S_C</i>) | N/A | 125.968 | 0 |
| (<i>S_P</i> , <i>R_C</i> , <i>S_P</i> , <i>R_C</i>) 366d | N/A | 126.652 | 0 |
| (<i>R_P</i> , <i>R_C</i> , <i>R_P</i> , <i>R_C</i>) | 123.963 | 128.416 | 33 |
| (<i>R_P</i> , <i>R_C</i> , <i>R_P</i> , <i>S_C</i>) | 122.504 | 127.459 | 4 |
| (<i>R_P</i> , <i>R_C</i> , <i>S_P</i> , <i>S_C</i>) | N/A | 127.657 | 0 |
| (<i>R_P</i> , <i>R_C</i> , <i>S_P</i> , <i>R_C</i>) | N/A | 125.991 | 0 |
| ^a From a random 1296 | | | |

Table 34 Computed enthalpies of formation for all diastereomers of compound **366**.

In order to better correlate the configuration of the molecule with its likelihood to form intramolecular hydrogen bonding, a randomized search of the conformational preferences of the four diastereomers of dimeric compound **366** that would have arisen had we started the synthesis from **341b** was carried out. Again, computational data suggest that two of these diastereomers, with (*R_P*,*R_C*,*R_P*,*R_C*) and (*R_P*,*R_C*,*R_P*,*S_C*) configuration, are likely to form intramolecular hydrogen bonds whereas the other two with (*R_P*,*R_C*,*S_P*,*R_C*) and (*R_P*,*R_C*,*S_P*,*S_C*) configuration, are unlikely to form intramolecular hydrogen bonds. A similar trend was observed for diastereomers **365a-d** (Table 35) and **367a-d** (Table 36). This leads to an understanding that the propensity of dimeric α -hydroxyphenylphosphinates to form intramolecular hydrogen bonds directly correlates to the relative configurations at the carbon and phosphorus atoms.

| Relative Configuration | Lowest energy in H-bonded conformations (Kcal/mol) | Lowest energy in non H-bonded conformations (Kcal/mol) | Number of intramolecularly H-bonded conformations |
|--|--|--|---|
| (<i>S_P</i> , <i>R_C</i> , <i>R_P</i> , <i>R_C</i>) 365a | 158.210 | 160.882 | 10 |
| (<i>S_P</i> , <i>R_C</i> , <i>R_P</i> , <i>S_C</i>) 365b | 159.534 | 161.609 | 11 |
| (<i>S_P</i> , <i>R_C</i> , <i>S_P</i> , <i>S_C</i>) | N/A | 160.692 | 0 |
| (<i>S_P</i> , <i>R_C</i> , <i>S_P</i> , <i>R_C</i>) | N/A | 161.486 | 0 |
| (<i>R_P</i> , <i>R_C</i> , <i>R_P</i> , <i>R_C</i>) | 156.955 | 160.573 | 16 |
| (<i>R_P</i> , <i>R_C</i> , <i>R_P</i> , <i>S_C</i>) | 158.400 | 161.573 | 1 |
| (<i>R_P</i> , <i>R_C</i> , <i>S_P</i> , <i>S_C</i>) | N/A | 160.780 | 0 |
| (<i>R_P</i> , <i>R_C</i> , <i>S_P</i> , <i>R_C</i>) | N/A | 161.641 | 0 |
| ^a From a random 1152 | | | |

Table 35 Computed enthalpies of formation for all diastereomers of compound **365**.

| Relative Configuration | Lowest energy in H-bonded conformations (Kcal/mol) | Lowest energy in non H-bonded conformations (Kcal/mol) | Number of intramolecularly H-bonded conformations |
|--|--|--|---|
| (<i>S_P</i> , <i>R_C</i> , <i>R_P</i> , <i>R_C</i>) | 124.352 | 127.892 | 25 |
| (<i>S_P</i> , <i>R_C</i> , <i>R_P</i> , <i>S_C</i>) | 123.948 | 127.181 | 4 |
| (<i>S_P</i> , <i>R_C</i> , <i>S_P</i> , <i>S_C</i>) (367c) | N/A | 126.598 | 0 |
| (<i>S_P</i> , <i>R_C</i> , <i>S_P</i> , <i>R_C</i>) (367d) | N/A | 125.913 | 0 |
| (<i>R_P</i> , <i>R_C</i> , <i>R_P</i> , <i>R_C</i>) | 124.377 | 127.255 | 5 |
| (<i>R_P</i> , <i>R_C</i> , <i>R_P</i> , <i>S_C</i>) | 122.352 | 126.975 | 12 |
| (<i>R_P</i> , <i>R_C</i> , <i>S_P</i> , <i>S_C</i>) | N/A | 126.917 | 0 |
| (<i>R_P</i> , <i>R_C</i> , <i>S_P</i> , <i>R_C</i>) | N/A | 125.365 | 0 |
| ^a From a random 1152 | | | |

Table 36 Computed enthalpies of formation for all diastereomers of compound **367**.

2.4.4. X-ray analysis of α -hydroxy phenylphosphinate dimers

While the computational studies gave sufficient data that appeared to confirm the evidence obtained by NMR, it was decided to confirm the relative configurations of H-bonded and non H-bonded diastereomers. To achieve this aim, it was necessary to move towards determination of the structure of the molecules in the solid state by X-ray crystallography.

In the benzaldehyde series the first two diastereomers were both crystalline. The final two diastereomers were isolated as an oil mixture, therefore X-ray analysis was not possible. Based on the X-ray crystal structure **365a** had (S_P, R_C, R_P, R_C) relative configuration (**Figure 20**). The alignment of the atoms was not suitable for intramolecular hydrogen bonding to be possible, despite the solution studies and computational studies to back the theory up. It could be possible that the influence of the phenyl groups had an effect during crystallisation such that the orientation of the atoms would not permit hydrogen bonding.

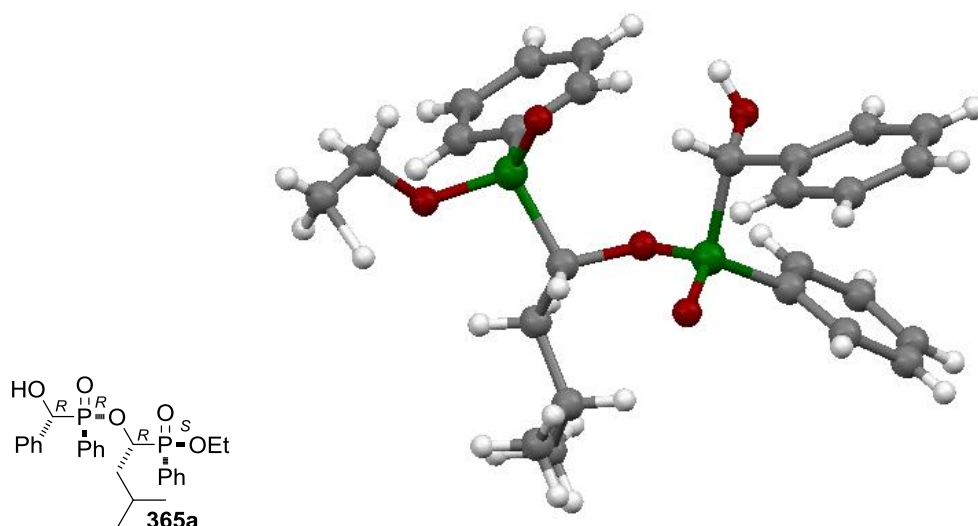


Figure 20 X-ray crystal structure of **365a**.

In the isovaleraldehyde series the first compound is an oil and the third one could not be crystallised. The remaining two diastereomers were crystalline and the X-ray crystal structures were obtained. Based on the results of the X-ray crystal structures **366b** had (S_P, R_C, R_P, S_C) relative configuration (**Figure 21**) and the alignment of the atoms suggested intramolecular hydrogen bonding between the hydrogen of the hydroxyl and the oxygen of the phosphonyloxy (P=O) functionality.

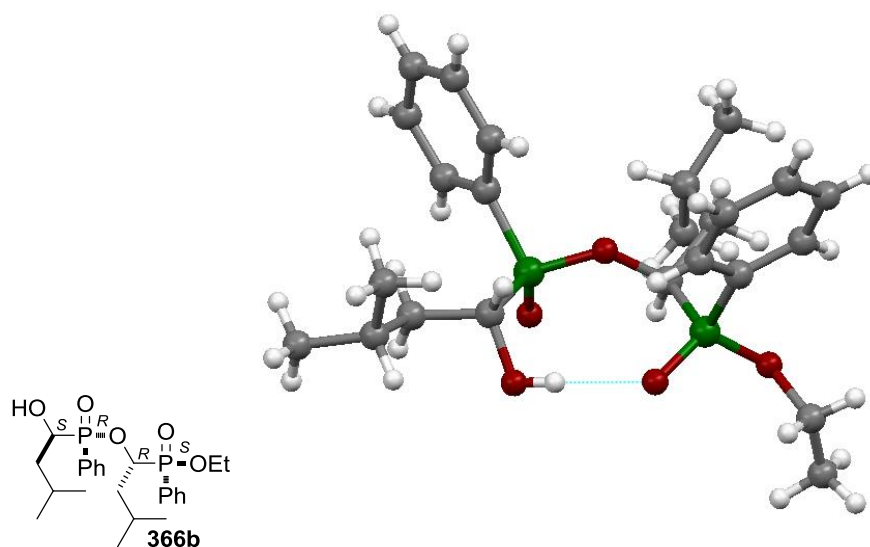


Figure 21 X-ray crystal structure of **366b**.

It was also possible to see that **366d** had (S_P, R_C, S_P, R_C) relative configuration (**Figure 22**) and the alignment of the atoms precluded any intramolecular hydrogen bonding.

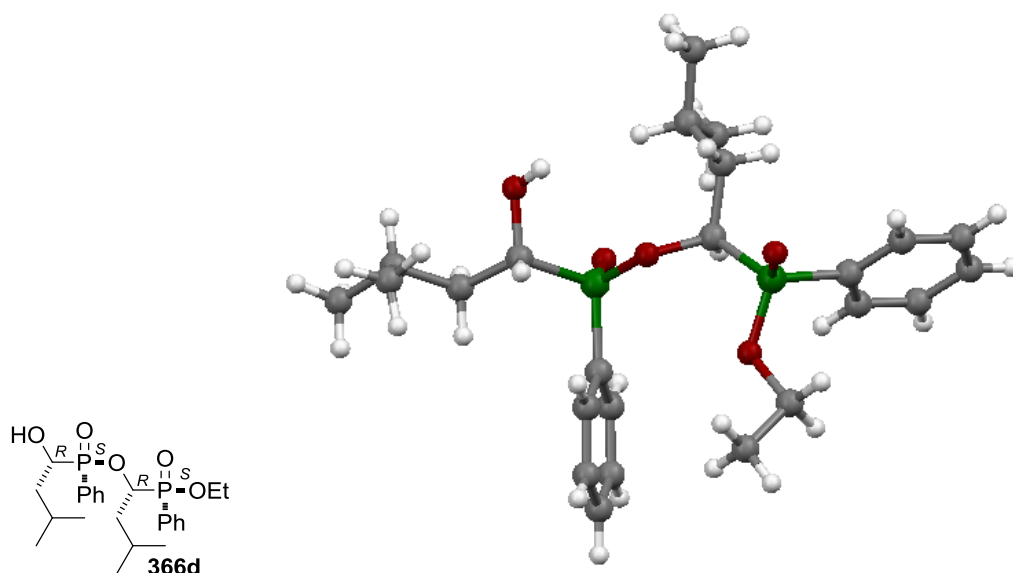


Figure 22 X-ray crystal structure of **366d**.

At this point some evidence was present to confirm the theories shown by solution and computational studies, however all of the information from one series of compounds had not been confirmed until looking at the other series of compounds formed from the reaction with isobutyraldehyde, dimer **367**.

In the isobutyraldehyde series all four diastereomers were crystalline. Based on the X-ray crystal structure **367a** had (S_P, R_C, R_P, R_C) relative configuration (**Figure 23**), with the alignment of the atoms suggesting intramolecular hydrogen bonding between the hydrogen of the hydroxyl and the oxygen of the phosphonyloxy ($P=O$) functionality.

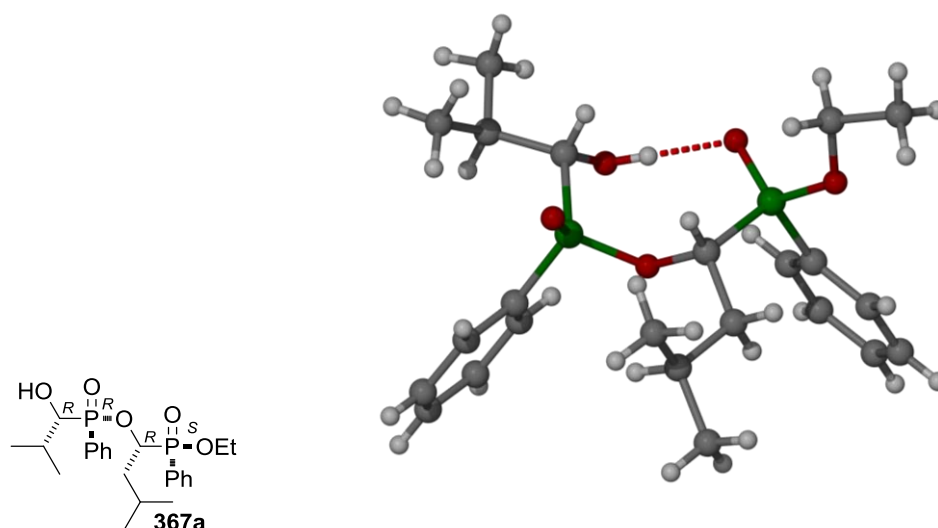


Figure 23 X-ray crystal structure of **367a**.

Based on the X-ray crystal structure **367b** had (S_P, R_C, R_P, S_C) relative configuration (**Figure 24**), with the alignment of the atoms suggesting intramolecular hydrogen bonding between the hydrogen of the hydroxyl and the oxygen of the phosphonyloxy (P=O) functionality.

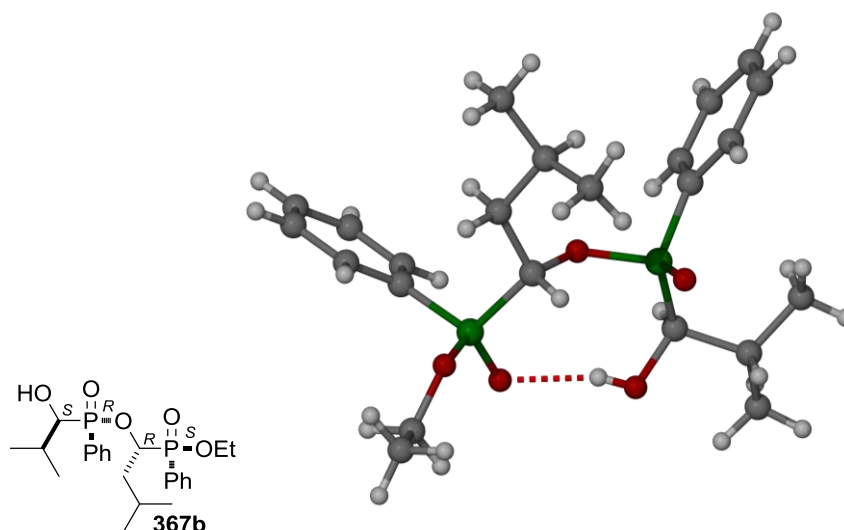
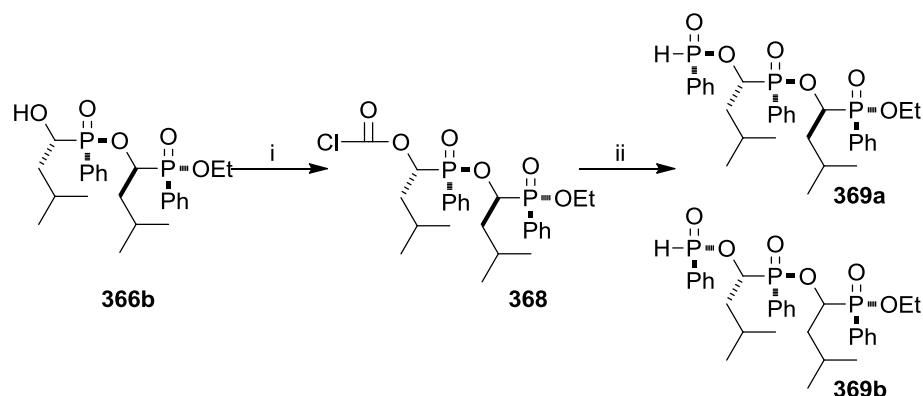


Figure 24 X-ray crystal structure of **367b**.

2.5. Synthesis of α -hydroxy phenylphosphinate trimers

It can clearly be seen that applying the Hewitt reaction followed by the phospho-aldol reaction sequentially could be carried out *ad infinitum*. In order to demonstrate the theory is applicable to synthesising longer oligomeric chains of α -hydroxy phenylphosphinate residues, chain elongation was carried out with one of the dimer α -hydroxy phenylphosphinates obtained in sufficient quantity.

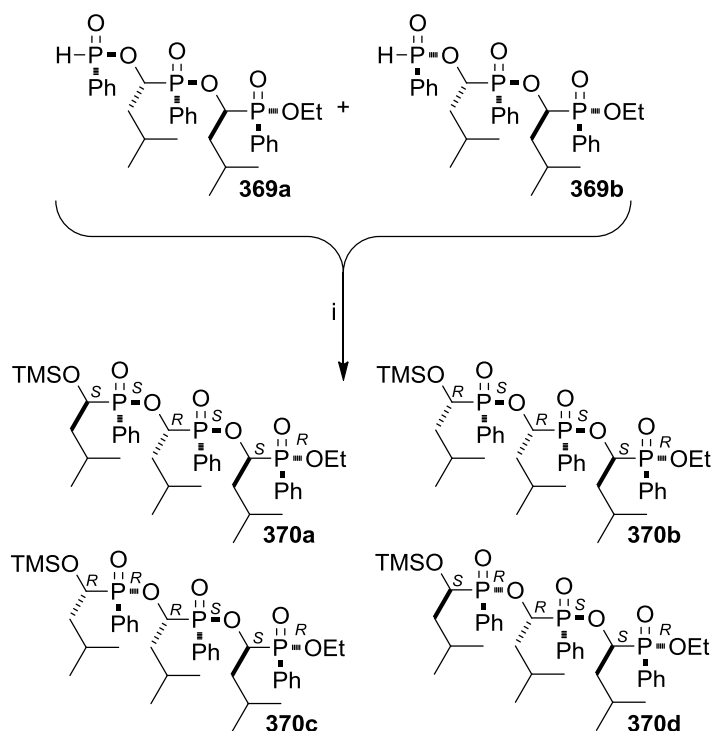
Compound **366b** was treated with triphosgene and pyridine in dichloromethane as shown in **Scheme 82**, to afford the corresponding chloroformate **368**. Once the chloroformate was formed it was treated under Hewitt reaction conditions to afford **369**. As with the synthesis of the dimer molecules the phosphite formed is a mixture of diastereomers that are tautomers, so isolation of the individual diastereomers was not possible at this stage.



Scheme 82 Reagents and conditions: (i) Py, (COCl)₂, CH₂Cl₂, 0 °C, 3 h; (ii) PhP(OH)₂, CH₂Cl₂, 40 °C, 1 h.

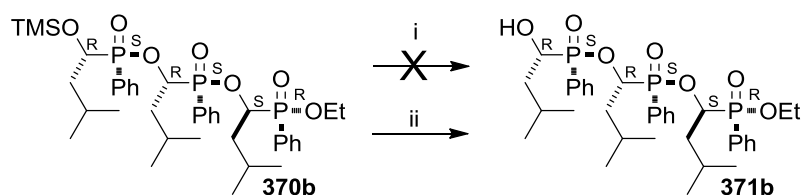
After phosphite **369a-b** was formed it could be used in the phospho-aldol reaction to form the trimer molecule (**Scheme 83**). The synthesis is carried out in the same manner as formation of the dimer molecules. The phosphite is first silylated to form the reactive intermediate, followed by treatment with isovaleraldehyde resulting in the formation of four diastereomeric trimer compounds **370a-d** (**Scheme 83**).

After the addition of the aldehyde and work up of the reaction the silyl protected alcohols were evident again as a mixture of four diastereomers **370a-d**. Separation was carried out and one of the diastereomers **370b** was isolated in pure form as an oil. The isolated diastereomer still had the TMS protecting group attached therefore deprotection had to be carried out to achieve the desired compound.



Scheme 83 Reagents and conditions: (i) Et₃N 15min, TMSCl 15min, isovaleraldehyde 0 °C to rt.

The first method attempted to deprotect the silyl group of the trimer (**Scheme 84**) was to treat it with camphorsulfonic acid, a weak acid, unfortunately this method for deprotection failed. To overcome this problem the trimer was immobilised on silica gel, left for 24 hours followed by extraction of the compound from the silica gel which gave the desired deprotected trimer compound **371b**.



Scheme 84 Reagents and conditions: (i) camphorsulfonic acid; (ii) Silica (SiO₂), 24 h.

2.6. Summary

A method for the synthesis of dimeric α -hydroxy phenylphosphinates has been developed and is has been proven by X-ray, NMR and computational studies that depending on the relative configuration at the carbon and phosphorus atoms, the structures may fold over to form intramolecularly hydrogen bonded structures (**Figure 25**).

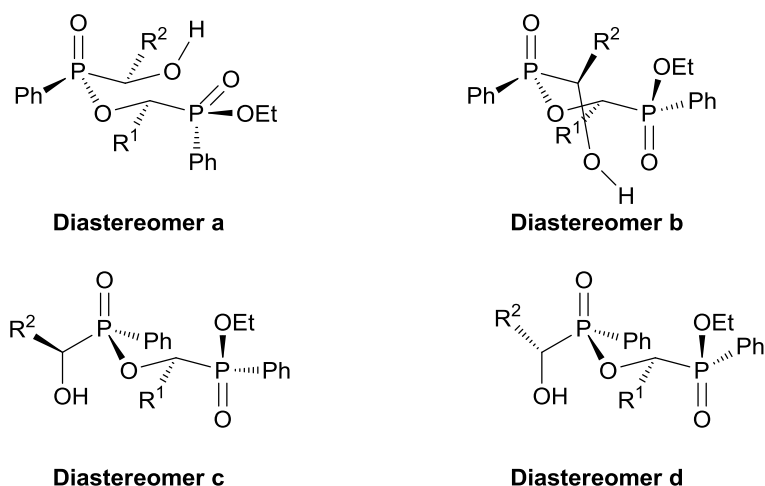
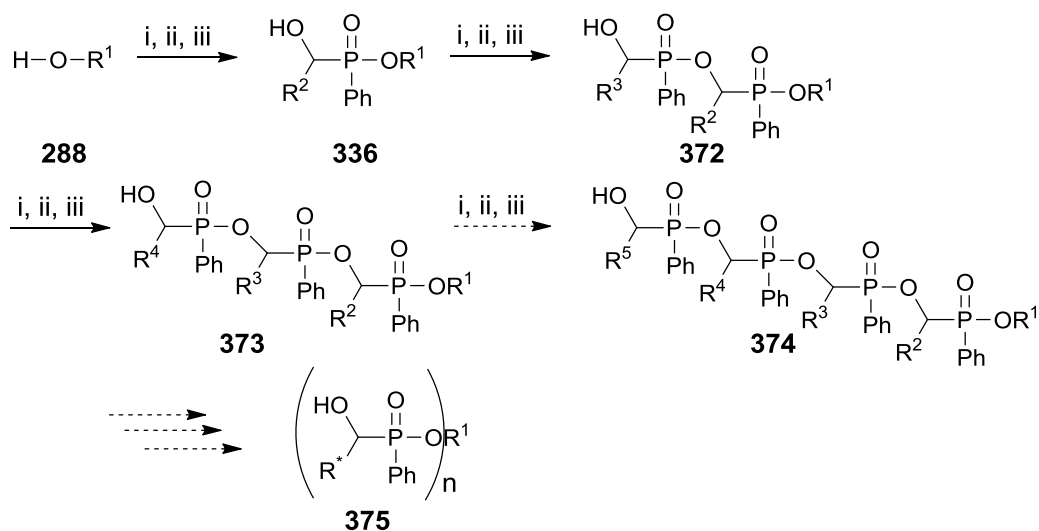


Figure 25 Structural conformations of dimer diastereomers.

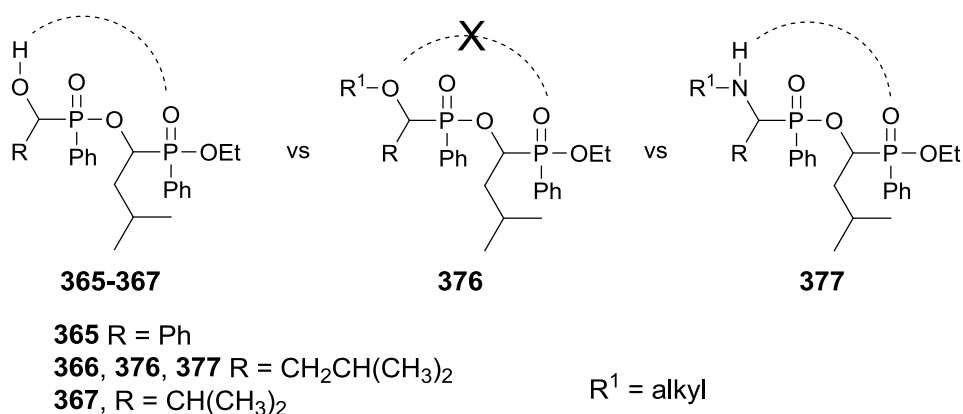
We have shown through the synthesis of that this methodology can be applied to longer chained oligomers (**Scheme 85**) by sequential chain extension through repeated chloroformate formation followed by Hewitt reaction and phospho-aldol reaction.



Scheme 85 (i) chloroformate formation; (ii) Hewitt reaction; (iii) phospho-aldol reaction

2.7. Synthesis of α -amino/ α -hydroxy phenylphosphinates

As part of an investigation into dimeric α -hydroxy phenylphosphinates (**Section 2.4**), we demonstrated that depending on the relative configuration of phosphorus and carbon atoms, these dimers fold over to give an eight-atom parsing intramolecular hydrogen bonding between the oxygen atom of the phosphonyl group (P=O) and hydrogen atom of the hydroxyl group (OH).



Scheme 86 Comparison of α -hydroxy, functionalised α -hydroxy and α -amino phenylphosphinates.

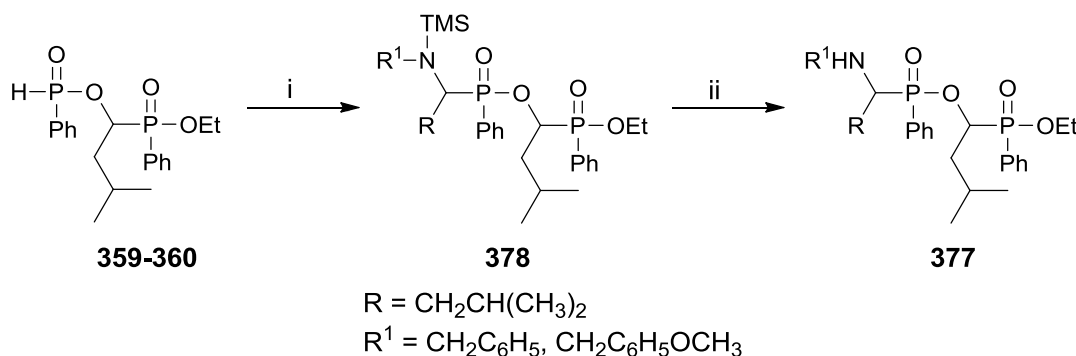
Although this was a fascinating observation, dimeric phenylphosphinate esters do not provide us with any further opportunity to investigate folding patterns by intramolecular H-bonding in more complex systems. Further substitution of the OH in these compounds also removes the hydrogen and the potential for H-bonding. Therefore, we decided to investigate the preparation of α -amino / α -hydroxy dimeric phenylphosphinates (**Scheme 86**). In these molecules there will still be a potential for intramolecular H-bonding with the remaining NH whilst other H-bond acceptor donors can be incorporated into the R group.

2.7.1. Via phospho-aldol reaction with imines

It was first decided to carry out the synthesis of the α -amino phenylphosphinates following a similar procedure to formation of the α -hydroxy phenylphosphinate dimers (**365-367**). The reaction involves transformation to the silyl protected intermediate,

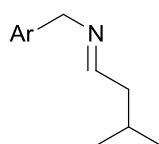
followed by the phospho-aldol reaction with an imine instead of the aldehyde. The reaction mechanism is as shown in **Scheme 63**.

The general scheme for synthesis of the α -amino phenylphosphinate dimers is shown below (**Scheme 87**):



Scheme 87 Reagents and conditions: (i) Et_3N 15 min, TMSCl 15 min, $\text{R}^1\text{N}=\text{CHR}$ 0°C to rt, (ii) aq. work-up.

The method for synthesis of α -amino phenylphosphinates involves reacting phosphite **359-360** with the desired imine. Formation of the imines was carried out according to **Scheme 73** to give **379** and **348** (**Figure 26**).



379 Ar = C_6H_5 quant.
348 Ar = $\text{C}_6\text{H}_4(4\text{-OMe})$ quant.

Figure 26 Imines synthesised for reaction with **359-360**.

Imines **379** and **348** were not purified, but reacted directly with phosphite **359-360** in the next step of the synthesis (**Scheme 87**). As in the synthesis of α -hydroxy phenylphosphinate dimers the reaction afforded four diastereomeric products **380a-d** and **381a-d** (**Figure 27**).

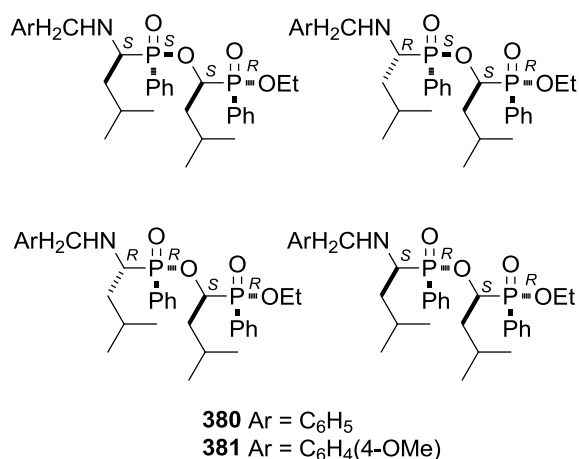
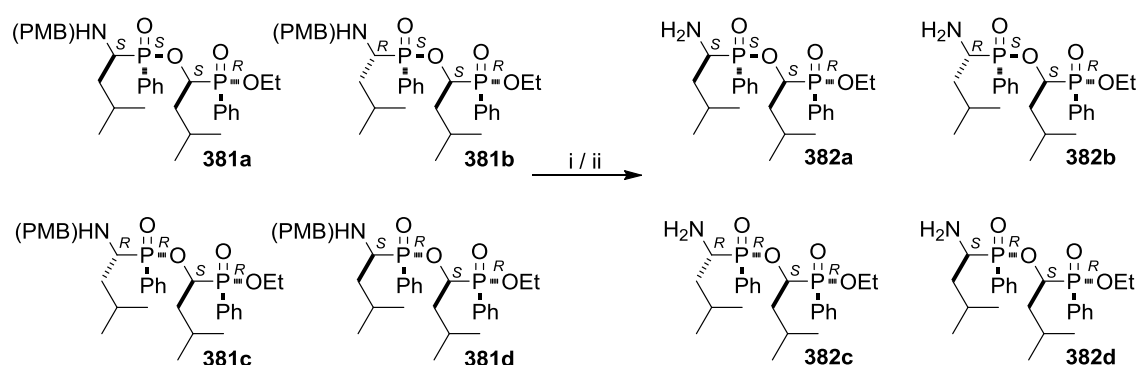


Figure 27 Protected α -amino phenylphosphinates synthesised.

Unfortunately separation of all of diastereomers **380a-d** was not possible. Diastereomers **381a-d** were part of a complex reaction mixture from which no single diastereomeric compound could be isolated was not possible at this stage. In an attempt to overcome this problem it was decided to remove the protecting group to give the free amine compound **382a-d**. Unfortunately removal of the protecting group with DDQ and hydrogenolysis (**Scheme 88**) did not completely remove the protecting group. Attempts to separate **381a-d** from **382a-d** as the reaction mixture was too complex to isolate a single diastereomeric compound.



Scheme 88 Reagents and conditions: (i) DDQ, CH₂Cl₂; (ii) Pd/C, H₂, EtOAc.

At this point further efforts towards the synthesis of α -amino phenylphosphinates using the phospho-aldol method were halted in favour of derivatisation of α -hydroxy phenylphosphinates.

2.7.2. Conversion of the hydroxyl group to azide

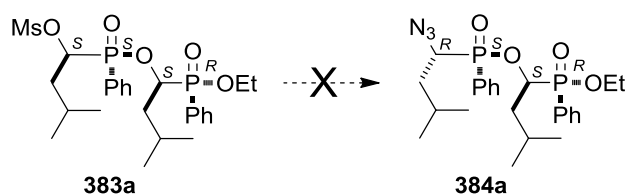
Since direct access to the *diastereomerically pure* α -amino phenylphosphinate through a phospho-aldol reaction to imines had proved to be problematic, it was decided to use an alternative approach. We had previously shown successfully, that the α -hydroxy phenylphosphinate monomer **347** could be converted to the corresponding α -amino phenylphosphinate **354a** via an azide displacement of the alcohol function, followed by reduction of the azide function (**Scheme 74** and **Scheme 75**). Therefore, this approach was adopted for *diastereomerically pure* α -hydroxy phenylphosphinate dimers.

The first step was to devise a method for conversion of the alcohol to an azide which could then be reduced to the corresponding amine. The first attempt was to convert the single diastereomeric compound **366b** to its corresponding tosyl derivative prior to reaction with sodium azide. This approach had previously been successfully applied to the synthesis of α -amino phenylphosphinate monomer **354a** (**Scheme 74**).

| No. | Starting Material | Reagents | Outcome |
|-------------|-------------------|---|-------------|
| 383b | 366b | TsCl, pyridine | No reaction |
| 383b | 366b | TsCl, Et ₃ N | No reaction |
| 383b | 366b | MsCl, pyridine, CH ₂ Cl ₂ | No reaction |
| 383b | 366b | MsCl, Et ₃ N, CH ₂ Cl ₂ | No reaction |
| 383a | 366a | Excess MsCl, Et ₃ N, CH ₂ Cl ₂ | Reaction |

Table 37 Conditions for leaving group formation.

This reaction did not proceed despite multiple attempts using excess of tosyl chloride and different bases. Attempts to synthesise the mesylate of **383a** were however successful. It was found that using a large excess of methanesulfonyl chloride and triethylamine, afforded the desired mesylate that could be purified by flash chromatography. The conditions used to form the mesylate are outlined in **Table 37**. However, when mesylate **383a** was treated with sodium azide the displacement reaction did not proceed (**Scheme 89**).



Scheme 89 Reagents and conditions: (i) NaN_3 , DMF, 60 °C, overnight.

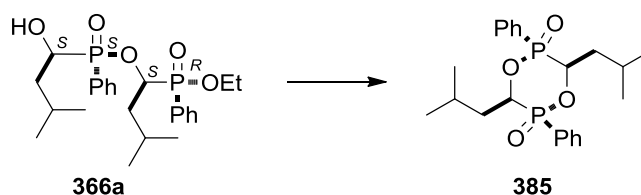
After failure to form azide **384a** from mesylate **383a** it was decided to convert alcohol **366a** to azide **384a** directly using Mitsunobu reaction (**Scheme 90**). Attempts for this reaction are shown in **Table 38**. This approach had previously been successfully applied to the synthesis of α -amino phenylphosphinate monomer **354a** (**Scheme 75**).

| No. | Reagents | Outcome |
|-------------|---|-------------|
| 366a | 1 eq. NaN_3 , 1eq. PPh_3 , DEAD, DMF | No reaction |
| 366a | 1 eq. NaN_3 , 2 eq. PPh_3 , DMF, CCl_4 | No reaction |
| 366a | 1 eq. NaN_3 , 5 eq. PPh_3 , DMF, CCl_4 | Reaction |

Table 38 Conditions used for azide formation. (Structure of **366a** shown in **Scheme 90**).

Initial attempts at the formation of azide **384a** from alcohol **366a** were unsuccessful, therefore an analogous method using sodium azide and triphenylphosphine and carbon tetrachloride in DMF was attempted as shown in **Table 38**. This resulted in a clean conversion (TLC) to a new product which was isolated by column chromatography.

After isolation of the product, NMR analysis clearly demonstrated that this was not the expected azide **384a**. In the ^1H NMR spectrum of the product the signals of the CH_3 and CH_2 of the ethyl ester were absent. The mass spectrum also did not show a molecular ion peak consistent with formation of the azide.



Scheme 90 Reagents and conditions: see **Table 39**.

After further spectroscopic analysis, including 2D NMR experiments, and full characterisation of the compound it was proposed that the product of the reaction was a 1,4,2,5-dioxadiphosphinane-2,5-dioxide ring, a cyclised version of starting alcohol **366a** (Scheme 90). The structure of compound **385** was subsequently confirmed by X-ray crystallography (Figure 28). X-ray crystallography also confirmed that the configuration of the carbon atom attached to the alcohol had been inverted during the reaction. The compound has a boat configuration, with one of the phenyls and two *i*-butyl groups in the pseudo equatorial position.

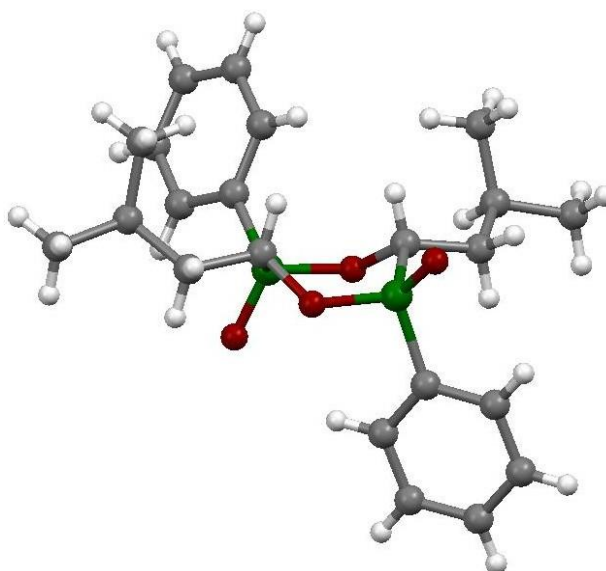
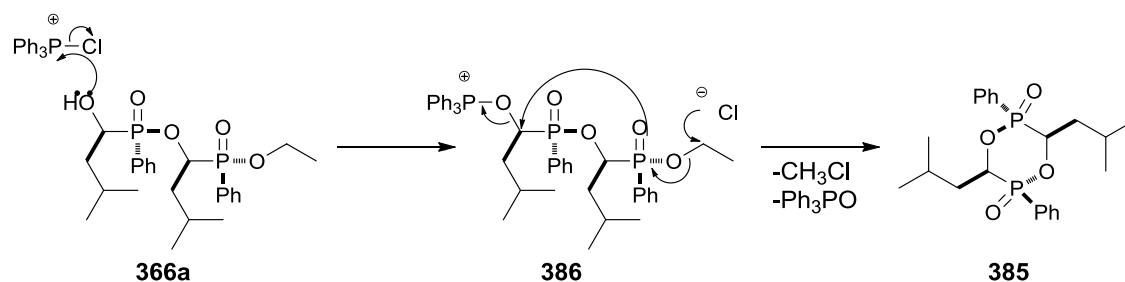


Figure 28 X-ray crystal structure of **385**

The mechanism below (Scheme 91) was proposed to explain how cyclisation of the alcohol could have taken place. Whilst it is a somewhat unusual mechanism, there are similar reactions involving cyclisation through the oxo oxygen atom of a phosphonyl moiety reported in the literature.^{96,97,98}



Scheme 91 Mechanism of cyclisation.

Interestingly, although 1,4,2,5-dioxadiphosphinane-2,5-dioxide ring has been previously synthesized and analysed by X-ray crystallography⁹⁹, this was the first ever example that could be applied to the synthesis of non-symmetric analogues. Therefore, we decided to briefly investigate the reaction. In an attempt to confirm the mechanism of the cyclisation, we varied the reaction conditions and reagents to investigate their role (**Table 39**).

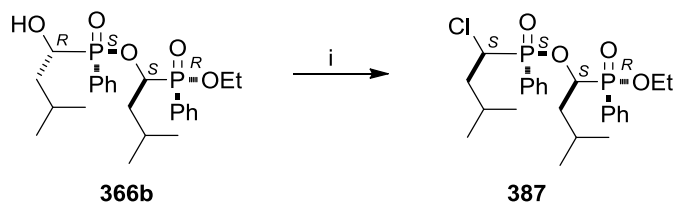
| Reagents | Outcome |
|--|-------------|
| NaN ₃ , PPh ₃ , CCl ₄ , DMF | Cyclisation |
| NaN ₃ , PPh ₃ , DMF | No reaction |
| PPh ₃ , CCl ₄ | Cyclisation |
| CCl ₄ | No reaction |
| PPh ₃ , DMF | No reaction |

Table 39 Varied reaction conditions for cyclisation (equivalents, time, and temperature are constant).

It could be seen from the investigations outlined in (**Table 39**) that the cyclisation reaction did not require sodium azide or DMF but it did require triphenylphosphine and carbon tetrachloride as predicted by the proposed mechanism.

Rather unexpectedly, we found that when diastereomer **366b** was subjected to the same reaction conditions that caused the transformation of **366a** to **385**, the analogous reaction did not proceed. Instead we obtained chloride **387** with inversion of stereochemistry (**Scheme 92**). The reason for this difference is not very clear but presumably is related to steric condition in the intermediates for the intramolecular

cyclisation in **366b**. The generality of the cyclisation is currently under further investigation.

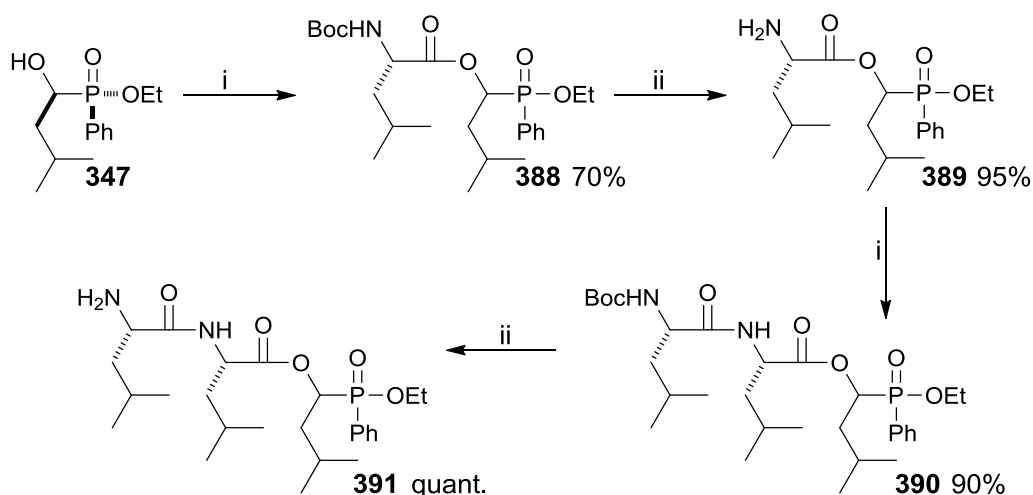


Scheme 92 Reagents and conditions: (i) 10 eq. PPh₃, CCl₄, 60 °C, 3 h.

2.8. Coupling amino acids to the phenylphosphinate analogue of leucine

2.8.1. Couplings to α -hydroxy phenylphosphinates

After success with the preparation of dimeric phenylphosphinates, it was decided to investigate the preparation of pseudopeptides with a phenylphosphinate at the C terminus. As outlined before (**Figure 14**), it was envisaged that at least two amino acid residues would need to be coupled before any folding patterns could be observed. This could be achieved either *via* stepwise coupling of two leucine residues (as shown in **Scheme 93** and **Scheme 94**), or *via* coupling of a di-leucine at the free hydroxyl or of phenylphospholeucine (as shown in **Scheme 95**).



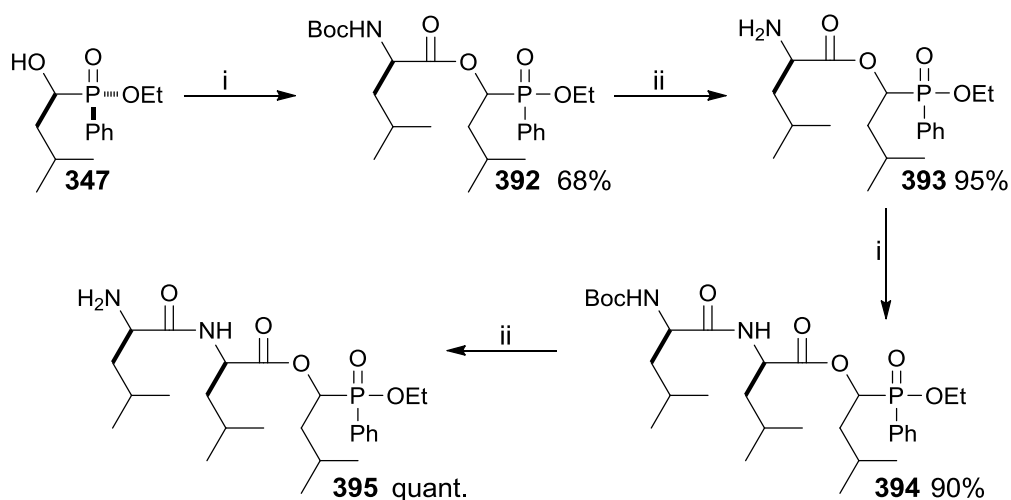
Scheme 93 Reagents and conditions: (i) DCC, DMAP, CH₂Cl₂, Boc-(L)-Leu-OH; (ii) TFA, CH₂Cl₂, 0 °C.

Compound **347** was coupled to Boc-(L)-Leu-OH using DCC and DMAP as coupling reagents (**Scheme 93**). The coupling gave **388** in quantitative yield as shown by NMR analysis; however the isolated yield was only 70%. This could be associated with problematic column chromatography of a polar, acid sensitive **388**. The coupling afforded two diastereomeric products as expected from the reaction of an enantiomer mixture of the starting alcohol **347** with an enantiopure reactant. The two diastereomers formed from the coupling could not be separated as they showed as a single spot on TLC. The mixture of diastereomers was solid and recrystallisation to isolate single diastereomers was attempted, however separation of diastereomers by this method was unsuccessful. The next step was to remove the Boc group hoping that the diastereomers of the free amine may be separable. The Boc protecting group is acid labile, therefore treatment of **388** with neat trifluoroacetic acid removed the protecting group to give the free amine **389** as expected. The products isolated were again a mixture of diastereomers that unfortunately could not be separated by column chromatography or crystallisation (**Scheme 93**).

Despite the inability to separate the diastereomers from each other, it was decided to continue the chain extension to add another leucine residue to **389** using the same

method previously discussed (**Scheme 93**) for formation of **388** to form **390**. The reaction proceeded with a good yield giving four diastereomeric products. Unfortunately as with the first coupling reaction no single diastereomer could be isolated. Again in an attempt to isolate a single diastereomer the Boc protecting group was removed. This reaction was carried out under the same conditions as the deprotection of **388** to give **391**. The reaction proceeded with quantitative yield but after work up and purification no single isomer could be obtained from the mixture of diastereomers.

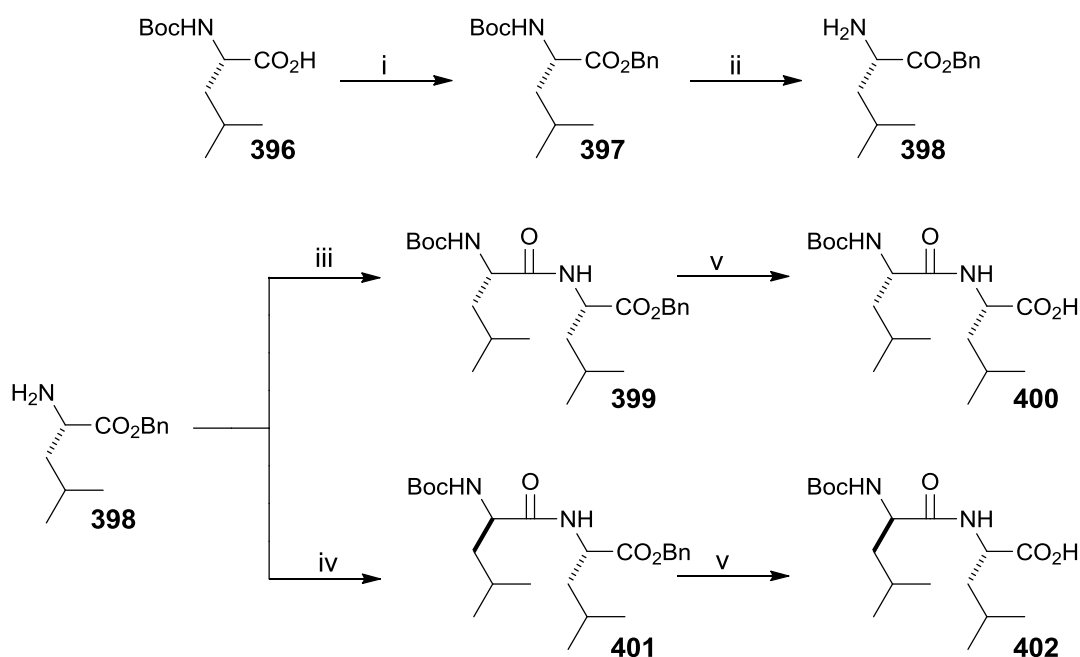
The coupling of **347** with Boc-(D)-Leu-OH was also attempted (**Scheme 94**) to afford **392** followed by deprotection of the Boc group to give **393**. As before coupling of **393** was carried out to give **394** followed by deprotection to give **395**. As previously the diastereomers were not separable by purification.



Scheme 94 Reagents and conditions: (i) DCC, DMAP, CH₂Cl₂, Boc-(D)-Leu-OH; (ii) TFA, CH₂Cl₂, 0 °C.

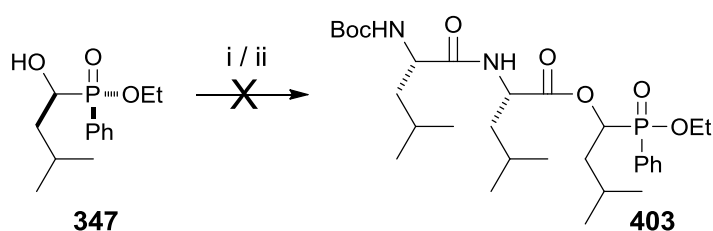
Due to problems with separation it was decided to couple diamino acids **400** and **402** (**Scheme 95**) to monomer **347** (**Scheme 96**, **Scheme 97**). The reaction was carried out using peptide conditions.

The synthesis of the diamino acids **400** and **402** is outlined in **Scheme 95**.

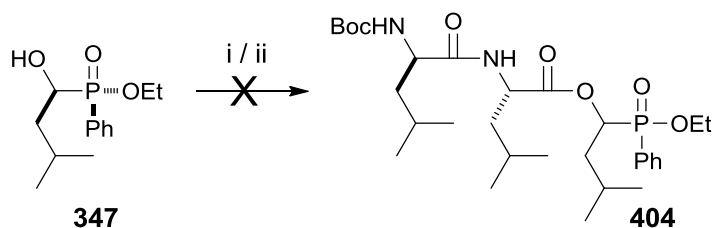


Scheme 95 Reagents and conditions: (i) Cs_2CO_3 , DMF, 60 °C, 1 h; then BnBr (0 °C for addition) 60 °C, 3 h; (ii) TFA, CH_2Cl_2 , 0 °C; (iii) DCC, DMAP, CH_2Cl_2 , Boc-(L)-Leu-OH; (iv) DCC, DMAP, CH_2Cl_2 , Boc-(D)-Leu-OH; (v) Pd/C 5%, H_2 , EtOAc.

After formation of diamino acids **400** and **402**, coupling was attempted to alcohol **347** using peptide coupling conditions (**Scheme 96** and **Scheme 97**), however the couplings did not proceed.



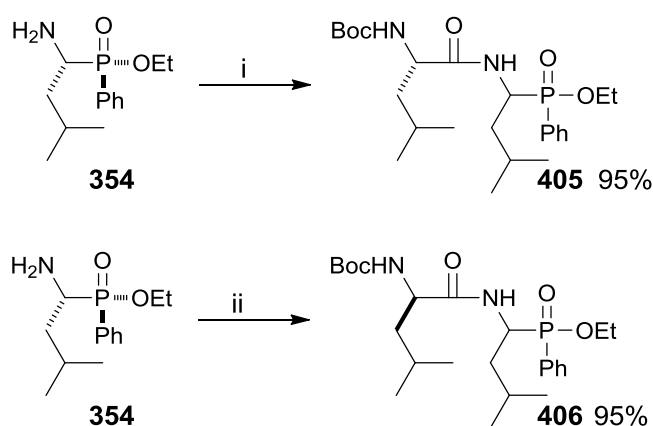
Scheme 96 Reagents and conditions: (i) HBTU, Et_3N , **400**, THF; (ii) DCC, DMAP, **400**, CH_2Cl_2 .



Scheme 97 Reagents and conditions: (i) HBTU, Et_3N , **402**, THF; (ii) DCC, DMAP, **402**, CH_2Cl_2 .

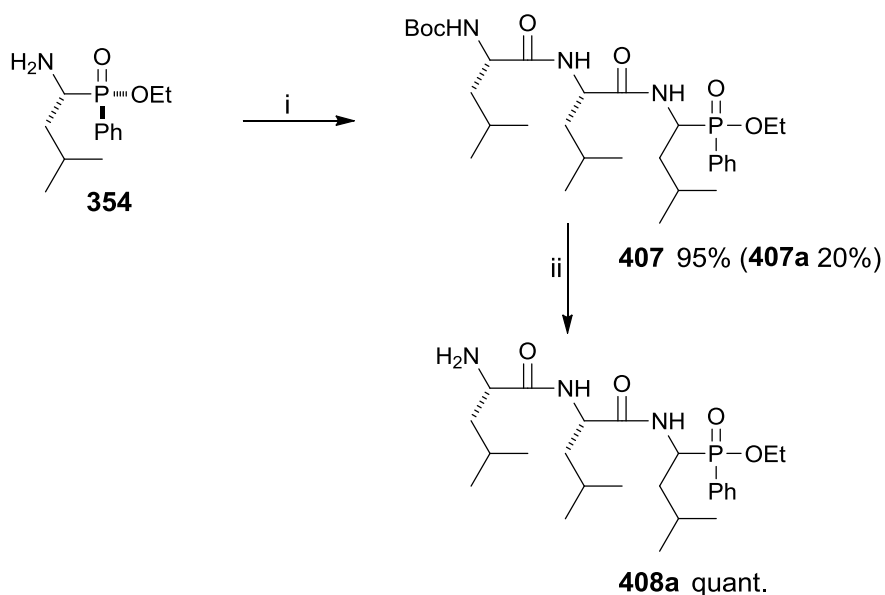
2.8.2. Coupling to α -amino phenylphosphinates

Although the attempted syntheses were mostly successful, any further progress was hampered by the fact that equal mixtures of diastereomers had been obtained. Because of this, NMR and other analysis of the mixtures were complex and unreliable. Therefore, it was decided to investigate coupling to α -amino phenylphosphinates, rather than α -hydroxy phenylphosphinates, in the hope that separation of the diastereomers, and hence the analysis of any supramolecular structures would become possible.



Scheme 98 Reagents and conditions: (i) HBTU, Et₃N, THF, Boc-(L)-Leu-OH; (ii) HBTU, Et₃N, THF, Boc-(D)-Leu-OH.

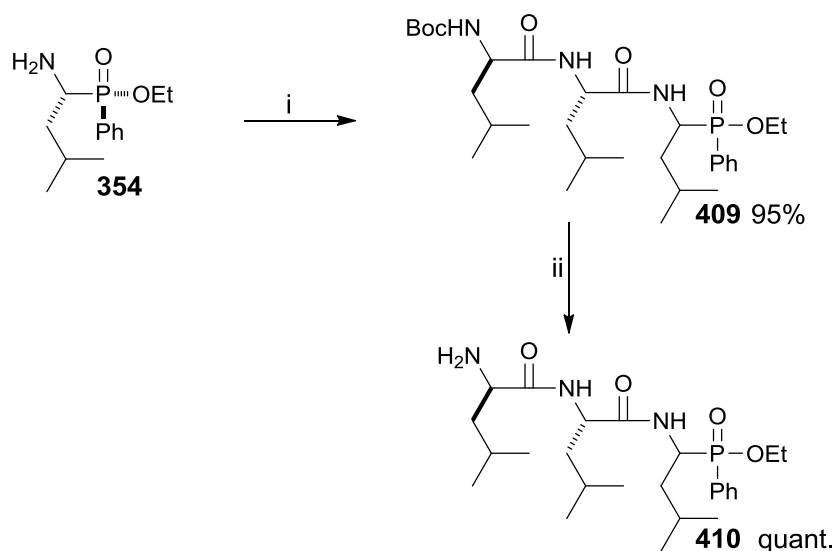
Using similar methods used in the couplings to α -hydroxy phenylphosphinates, α -aminophenylphosphinate **354a** was coupled to Boc-(L)-Leu-OH and Boc-(D)-Leu-OH using HBTU and triethylamine as the coupling reagents (**Scheme 98**). The reaction proceeded with good yield to afford **405** and **406** both as a mixture of diastereomers. In spite of attempts of separation, compounds **405** and **406** were not isolated as single diastereomers. For the synthesis of “trimers” we decided to directly couple diamino acids Boc-(L)-Leu-(L)-Leu-OH **400** and Boc-(D)-Leu-(L)-Leu-OH **402**, previously synthesised as outlined in **Scheme 95**, to the α -amino phenylphosphinate **354a**.



Scheme 99 Reagents and conditions: (i) HBTU, Et₃N, THF, **400**; (ii) TFA, CH₂Cl₂, 0 °C.

α -Amino phenylphosphinate **354a** was coupled to **400** using HBTU and triethylamine (**Scheme 99**) following the same procedure as described earlier (**Scheme 98**). The reaction gave two diastereomers one of which, **407a**, was isolated in pure form (20% yield). However the other diastereomer **407b** could not be separated in pure form and was always obtained as a mixture with the other diastereomer. Product **407a** was subsequently deprotected using TFA to give **408a**. Unfortunately product **408a** was obtained as an oil therefore crystallographic studies to determine the relative configuration of the molecule were not possible.

Careful analysis of the NMR spectra of **408a** however did not show any evidence to suggest any intramolecular hydrogen bonding in this molecule.



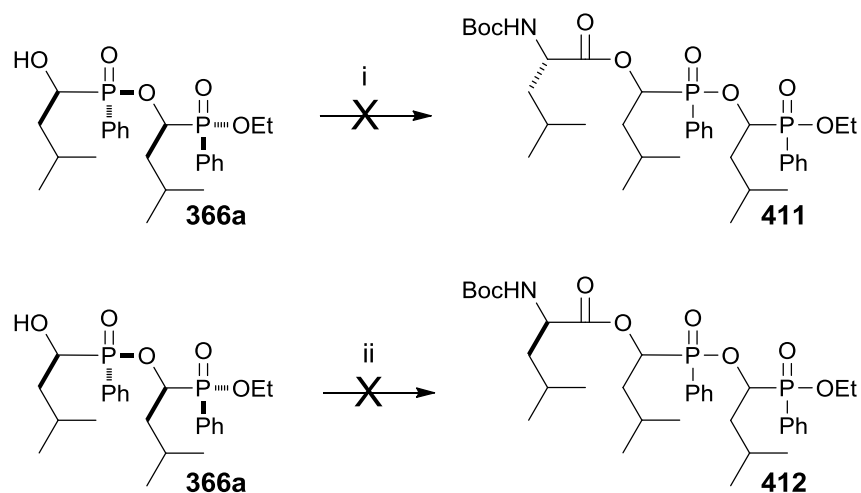
Scheme 100 *Reagents and conditions:* (i) HBTU, Et₃N, THF, **402**; (ii) TFA, CH₂Cl₂, 0 °C.

α -Amino phenylphosphinate **354a** was also coupled to **402** (Scheme 100) using the same procedure as described in Scheme 99. The reaction gave two diastereomers **409a-b** that could not be separated. The products **409a-b** were deprotected using TFA as before to give a crystalline mixture of diastereomers **410a-b**. Even though the diastereomers were not separable, the quality of the crystals were sufficiently high to encourage us to obtain X-ray crystallography, which is currently pending.

2.9. Coupling to leucine dimer analogue

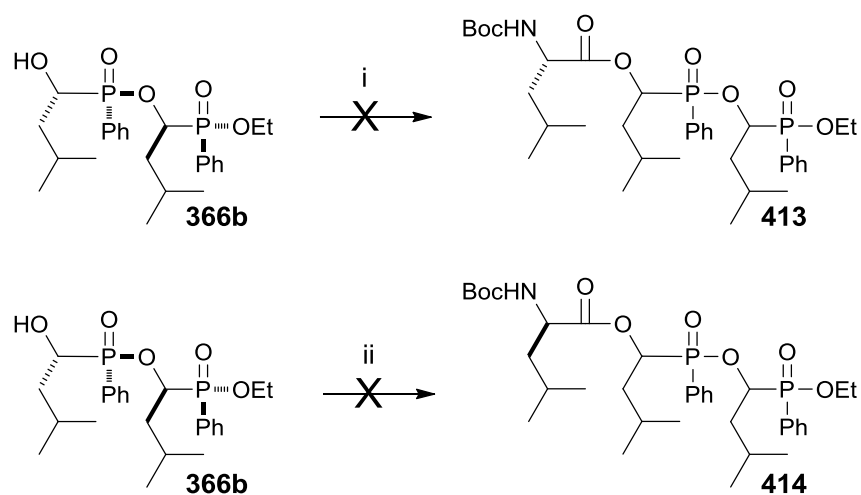
As separation of diastereomerically pure compounds of Leu-Leu-Leu(P) was proving very difficult and the investigation into the secondary structures were unrewarding, it was decided to change track slightly and investigate Leu-Leu(P)-Leu(P) trimers. Our success with Leu(P)-Leu(P), both for the separation of diastereomers as well as observation of folding patterns encouraged this exercise.

Compounds **366a** and **366b** were chosen to be coupled with protected amino acids.



Scheme 101 Reagents and conditions: (i) DCC, DMAP, CH₂Cl₂, Boc-(L)-Leu-OH; (ii) DCC, DMAP, CH₂Cl₂, Boc-(D)-Leu-OH.

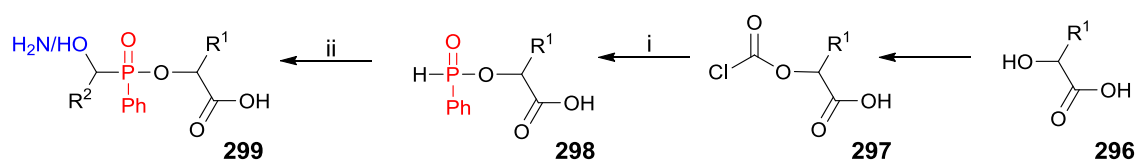
Compounds **366a** and **366b** were reacted with Boc-(L)-Leu-OH and Boc-(D)-Leu-OH using the conditions shown in **Scheme 101** and **Scheme 102**, however the coupling reaction did not proceed recovering only the starting materials.



Scheme 102 Reagents and conditions: (i) DCC, DMAP, CH₂Cl₂, Boc-(L)-Leu-OH; (ii) DCC, DMAP, CH₂Cl₂, Boc-(D)-Leu-OH.

2.10. Formation of α -hydroxy carboxylic acids

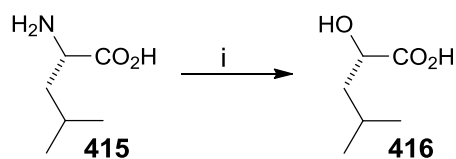
As discussed earlier (**Scheme 55**), to prepare phosphonopeptides with a phosphinic residue at the middle of the chain, it is possible to start from an α -hydroxy carboxylate **296**, convert it to the chloroformate **297**, then *via* the Hewitt reaction to the corresponding phenylphosphinate ester **298**, which can then be used in a phospho-aldol reaction to give **299** (**Scheme 103**).



Scheme 103 Reagents and conditions: (i) Hewitt reaction; (ii) phospho-aldol reaction.

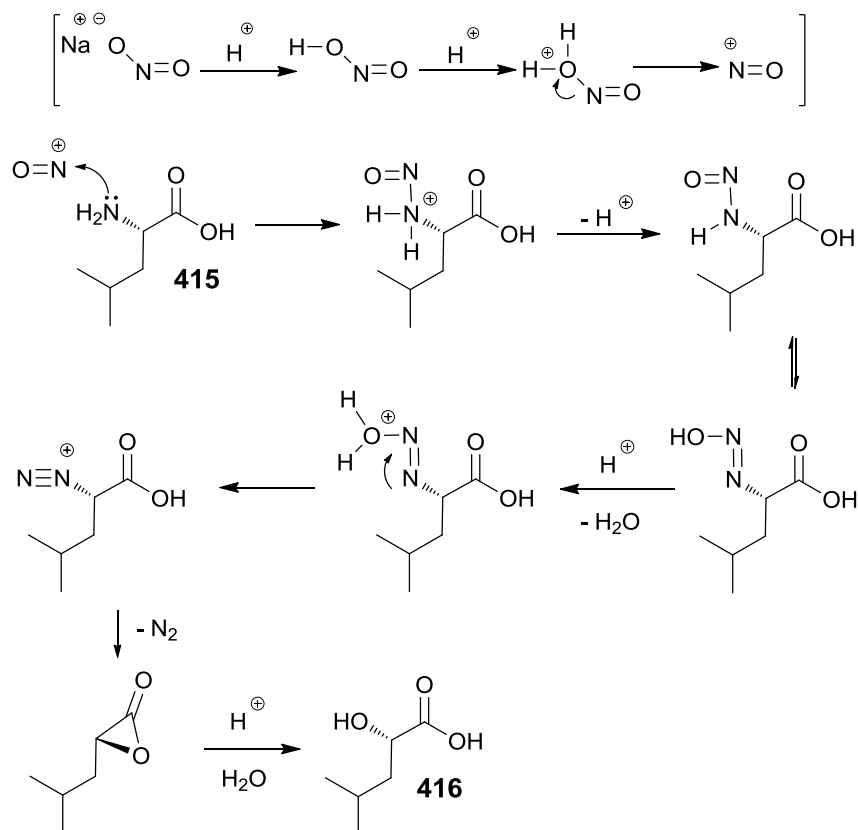
In view of our existing experience with leucine analogues, it was decided that the R¹ group should be an isobutyl group (**Scheme 103**), meaning that the synthesis could start from 2-hydroxy-4-methylpentanoic acid (hydroxy leucine).

2.10.1. Preparation of 2-hydroxy-4-methylpentanoic acid



Scheme 104 Reagents and conditions: (i) 0.5M H_2SO_4 , NaNO_2 , 0 °C to rt.

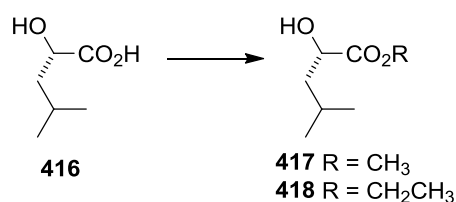
Conversion of (L)-leucine **415** to (S)-2-hydroxy-4-methylpentanoic acid **416** was achieved using a diazotization reaction (**Scheme 104**). Diazotization involves a double inversion at the carbon stereocenter. The mechanism for the conversion is shown in **Scheme 105**.



Scheme 105 Mechanism of conversion of amine to alcohol *via* diazotization.

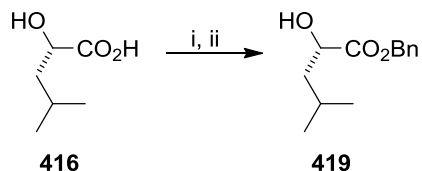
The enantiopurity of the alcohol was recorded using a polarimeter, obtaining a specific rotation of -27.4° , in agreement with -27.6° reported in the literature for the enantiopure compound.¹⁰⁰

The resulting α -hydroxy carboxylic acid was converted to its methyl (**417**) and ethyl (**418**) esters through an acid catalysed esterification (**Scheme 106**).



Scheme 106 Reagents and conditions: (i) AcCl , ROH , 0°C .

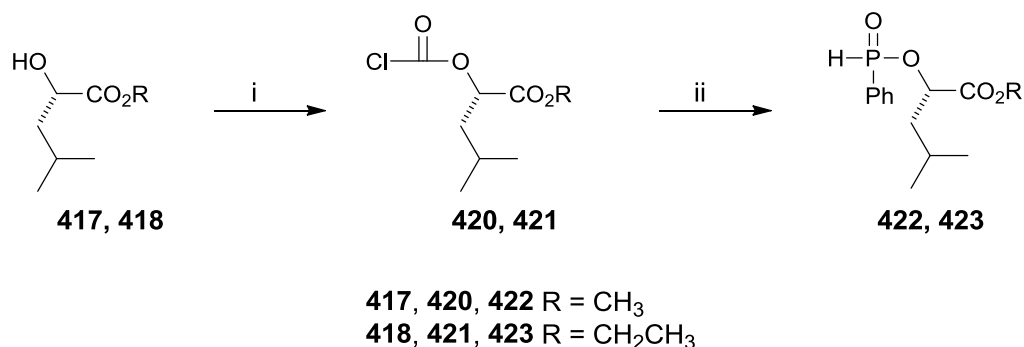
The selective formation of the benzyl ester **419** (without affecting the formation of a benzyl ether) can be achieved through use of a base such as cesium carbonate and benzyl bromide (**Scheme 107**).



Scheme 107 *Reagents and conditions:* (i) Cs_2CO_3 , DMF, 60 °C, 1 h; (ii) BnBr (0 °C for addition) 60 °C, 3 h.

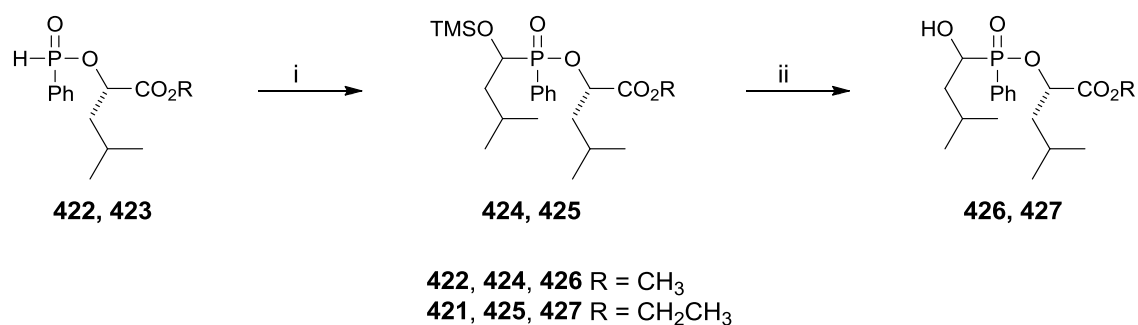
The next part of the synthesis requires formation of the phenyl phosphinate, which can be carried out over two steps, first forming the chloroformate then using Hewitt reaction conditions to form the corresponding phenyl phosphinate.

2.10.1.1. Reactions of phosphites derived from the methyl and ethyl esters



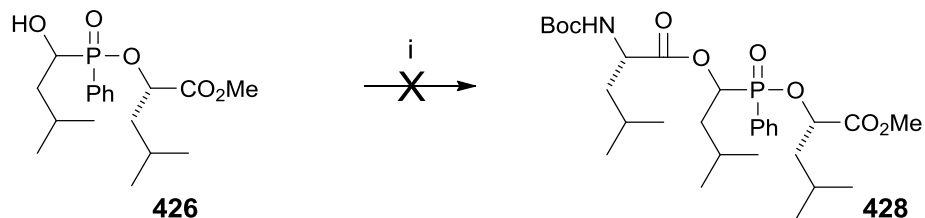
Scheme 108 *Reagents and conditions:* (i) Py, $(\text{COCl}_2)_3$, CH_2Cl_2 , 0 °C 3 h; (ii) $\text{PhP}(\text{OH})_2$, CH_2Cl_2 , 40 °C, 1 h.

Compounds **417** and **418** were reacted to form the corresponding chloroformates **420** and **421** (**Scheme 108**) followed by Hewitt reaction conditions to give the corresponding phenyl phosphinates **422** and **423** (**Scheme 108**).



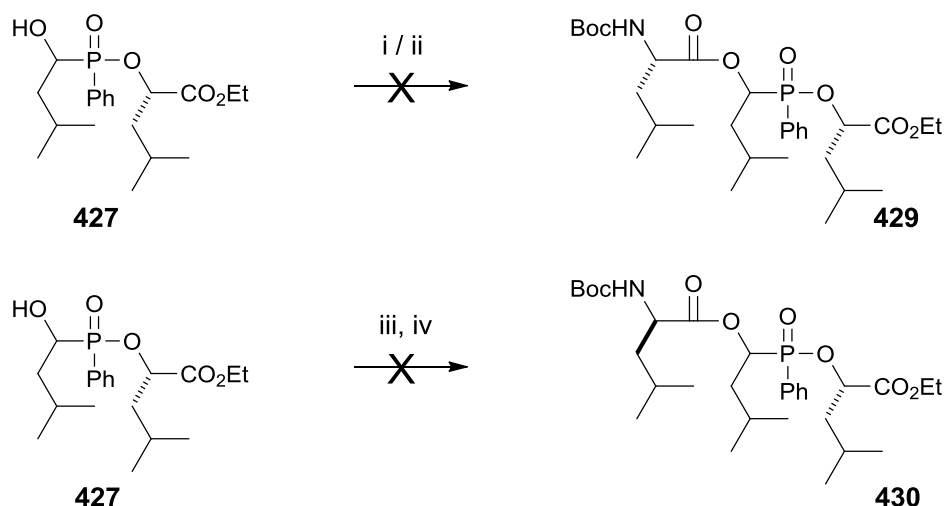
Scheme 109 *Reagents and conditions:* (i) Et₃N 15 min, TMSCl 15 min, isovaleraldehyde 0 °C to rt; (ii) TBAF, THF/Et₂O, 1 h.

Phosphites **422** and **423** were reacted with isovaleraldehyde to form **424** and **425** (**Scheme 109**). As was seen before, during the aqueous work up partial deprotection had taken place, therefore complete deprotection had to be carried out using TBAF to give **426** and **427**. Unfortunately, separation of the individual diastereomers in either of the ethyl or methyl series was not possible.



Scheme 110 *Reagents and conditions:* (i) Boc-(L)-Leu-OH, DCC, DMAP, CH₂Cl₂.

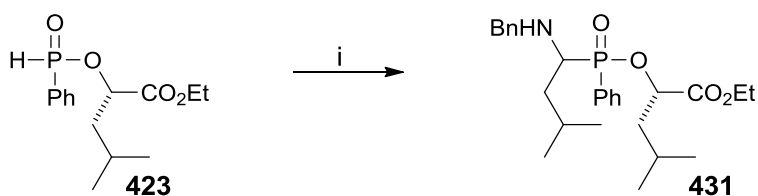
The coupling of methyl ester **426** to Boc-(L)-Leu-OH and of the ethyl ester **427** to both Boc-(L)-Leu-OH and Boc-(D)-Leu-OH were attempted but for reasons which were not clear, were unsuccessful (**Scheme 110** and **Scheme 111**).



Scheme 111 *Reagents and conditions:* (i) Boc-(L)-Leu-OH, DCC, DMAP, CH₂Cl₂; (ii) Boc-(L)-Leu-OH, HBTU, Et₃N, THF; (iii) Boc-(D)-Leu-OH, DCC, DMAP, CH₂Cl₂; (iv) Boc-(D)-Leu-OH, HBTU, Et₃N, THF.

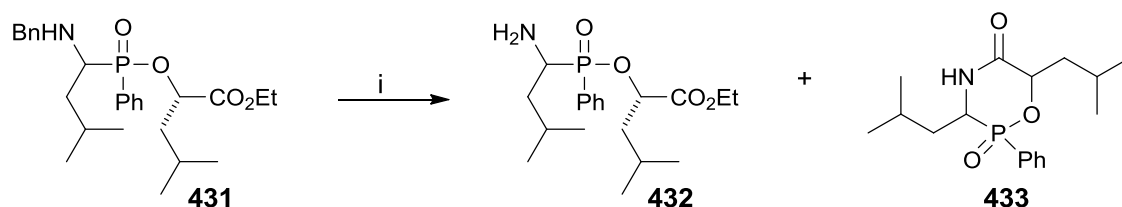
Similarly, conversion of the alcohol function in **427** to the azide function using methods previously employed, were unsuccessful (for comparison see **Scheme 75** and **Scheme 91**).

Therefore, the next step of the synthesis was to carry out the phospho-aldol reaction of phosphite **423** with imine **379**, to give protected amine **431** directly (**Scheme 112**).



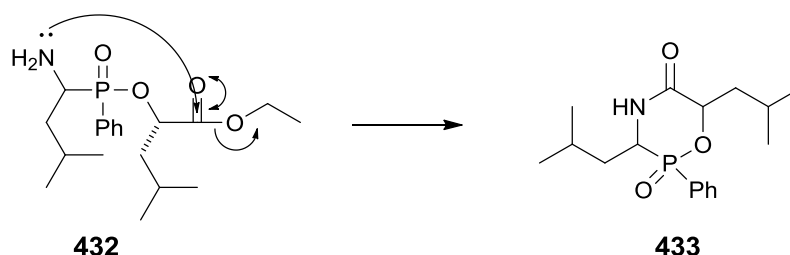
Scheme 112 *Reagents and conditions:* (i) Et₃N 15 min, TMSCl 15 min, imine 0 °C to rt.

The diastereomers of compound **431** could not be separated, however, the N-benzyl group was successfully removed under catalytic hydrogenation conditions (**Scheme 113**). Unfortunately though, free amine **432** was obtained with substantial quantities of a by-product **433** (**Scheme 113**) and therefore, this was not deemed a viable synthetic method.



Scheme 113 Reagents and conditions: (i) Pd/C 5%, H₂, EtOAc.

The ³¹P NMR of the crude reaction mixture showed that the four signals corresponding to the protected product **431** had disappeared, but eight new signals were present: four corresponding to the desired amine **432** with similar chemical shifts to the corresponding alcohol **427**, as well as four new signals at considerably different chemical shifts. Characterisation by NMR showed that the ethyl ester of the carboxylic acid had been removed and a secondary amide had been formed. Based on these observations, it was concluded that compound **432** had undergone an intramolecular cyclisation forming compound **433**.

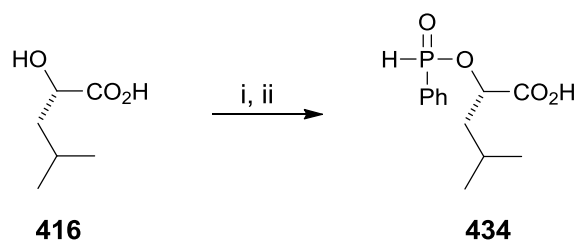


Scheme 114 Mechanism of cyclisation of **432**.

The mechanism proposed for the cyclisation reaction is shown in **Scheme 114**. It is thought that once the amine is formed under hydrogenation conditions the lone pair of the nitrogen attacks the carbonyl of the ester and cyclisation occurs.

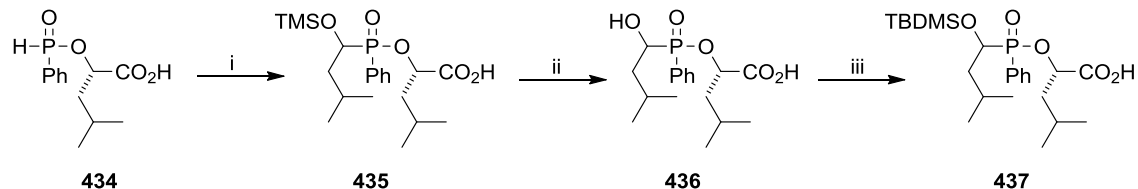
2.10.2. Modifications to the free carboxylic acid

In spite of numerous attempts to derivatise and purify the compounds mentioned, the results were not promising. To try and overcome this obstacle it was decided to attempt the synthesis on the free carboxylic acid.



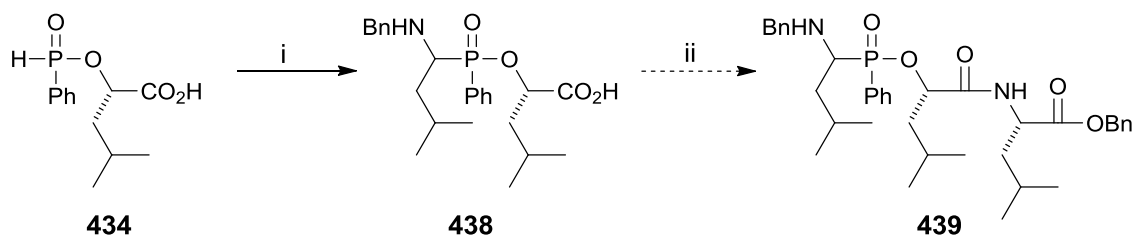
Scheme 115 *Reagents and conditions:* (i) Py, (COCl₂)₃, CH₂Cl₂, 0 °C 3 h; (ii) PhP(OH)₂, CH₂Cl₂, 40 °C, 1 h.

Compound **416** was successfully transformed to its phenylphosphinate derivative **434** through the reaction described in **Scheme 115**.



Scheme 116 *Reagents and conditions:* (i) Et₃N 15 min, TMSCl 15 min, isovaleraldehyde 0 °C to rt; (ii) TBAF, THF/Et₂O; (iii) TBDMSCl, imidazole, CH₂Cl₂.

Compound **434** was then reacted with isovaleraldehyde under silyl mediated phospho-aldol conditions to form compound **435**, which would then be desilylated to give compound **436** (**Scheme 116**). Compound **436** was part of a complex reaction mixture from which no single diastereomeric compound could be isolated was not possible at this stage. In an attempt to aid separation, compound **436** was converted to **437**, however the diastereomers were still inseparable.



Scheme 117 Reagents and conditions: (i) Et₃N 15min, TMSCl 15min, **379** 0 °C to rt; (ii) **396**, DCC, DMAP, CH₂Cl₂.

Compound **434** was also reacted with imine **379** to give the corresponding protected amine product **438** (Scheme 117). Separation did not give single diastereomers so a coupling to the carboxylic acid end was attempted using H₂N-(L)-Leu-OBn **398** to give **439**. Further modifications including deprotection of the amine are pending.

2.11. Towards the synthesis of a long chain leucine analogue pseudopeptide

At this stage, it was decided to consider a different approach to achieving our objective of demonstrating secondary structure motifs from hybrids of α-amino carboxylates and α-amino/α-hydroxy phenylphosphinates. Instead of building larger peptides starting from smaller, diastereomerically pure α-amino/α-hydroxy phenylphosphinates, longer chain units would be attacked at either end of a diastereomerically mixed α-amino/α-hydroxy phenylphosphinate unit. Obviously, a mixture of peptides would be obtained but the idea was that these larger peptides might be more easily separable because the ones with the “correct” stereochemistry would fold and hence would have different properties that would enable separation.

To form this structure two tetra leucine molecules would be attached at the either end of α-amino phenylphosphinate/hydroxyleucine dimer **436**. This would give decamer **440** (Figure 29) which is likely to form an α-helical structure.

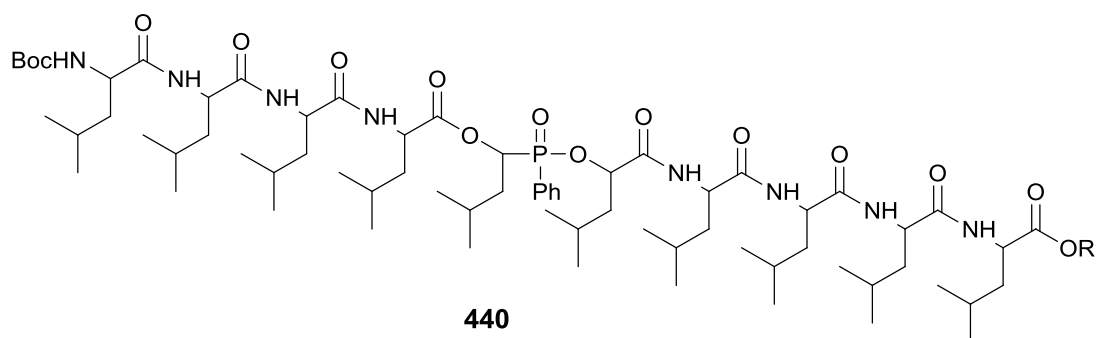
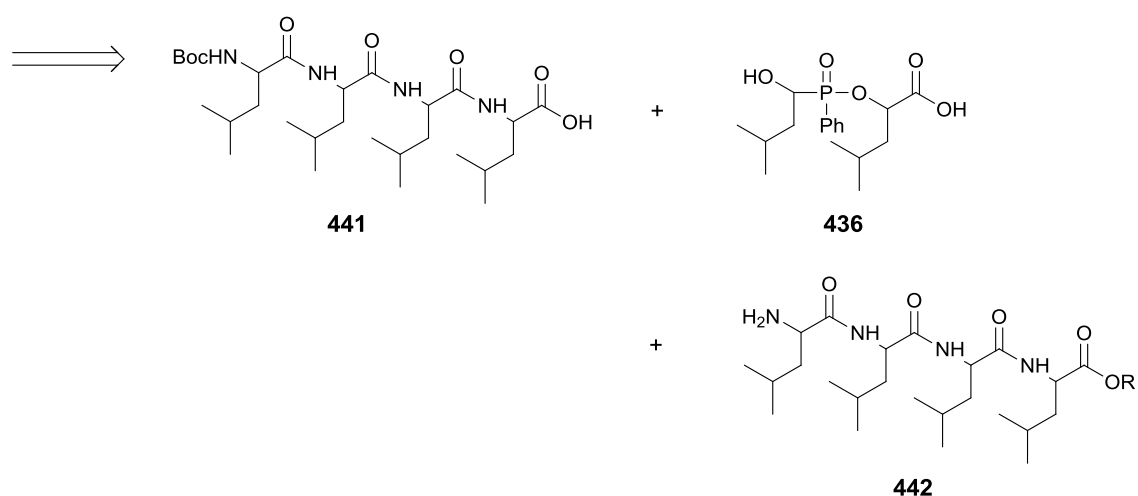


Figure 29 Proposed decamer to be synthesised.

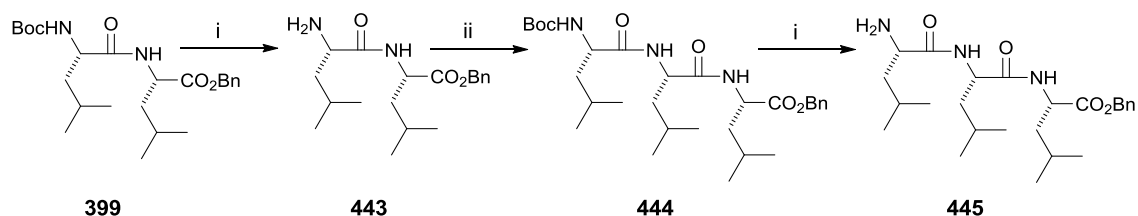
Pseudopeptide **440** can be divided into three parts that for synthesis (**Scheme 118**). Two components can be synthesised in the same manner, building up the tetramer of leucine then deprotecting either the C or N terminus followed by coupling to the central component. The remaining central component is α -hydroxy phenylphosphinate/hydroxyleucine dimer **436** (**Scheme 116**) which was previously synthesised.



Scheme 118 Retrosynthetic analysis of decamer **440**.

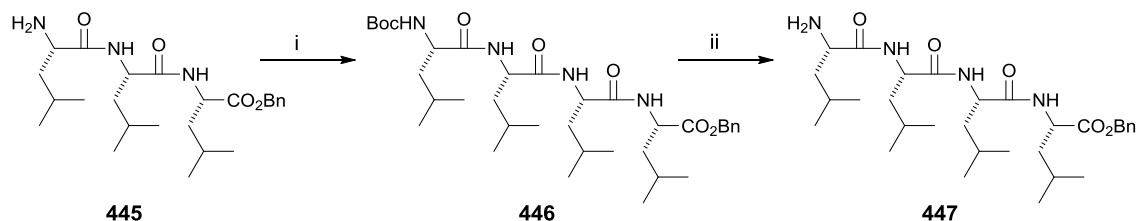
2.11.1. Synthesis of tetramer of leucine

The first component to be synthesised is the tetramer of leucine as this requires the most steps when carrying out a stepwise elongation of the peptide chain.



Scheme 119 Reagents and conditions: (i) TFA, CH₂Cl₂, 0 °C 30 min; (ii) Boc-(L)-Leu-OH, DCC, DMAP, CH₂Cl₂.

Starting from the previously synthesised diamino acid **399** (Scheme 95), three steps are carried out involving deprotection to give **443**, coupling to give **444** and a further deprotection using the same conditions as described previously to give the triamino acid **445** with the free N-terminus.

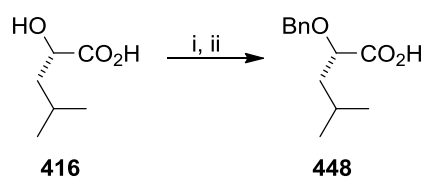


Scheme 120 Reagents and conditions: (i) Boc-(L)-Leu-OH, DCC, DMAP, CH₂Cl₂; (ii) TFA, CH₂Cl₂, 0 °C 30 min.

Continuing the synthesis from triamino acid **445**, two steps are carried out; a coupling reaction to give **446** followed by deprotection to give the benzyl ester of tetra leucine, compound **447** (Scheme 120).

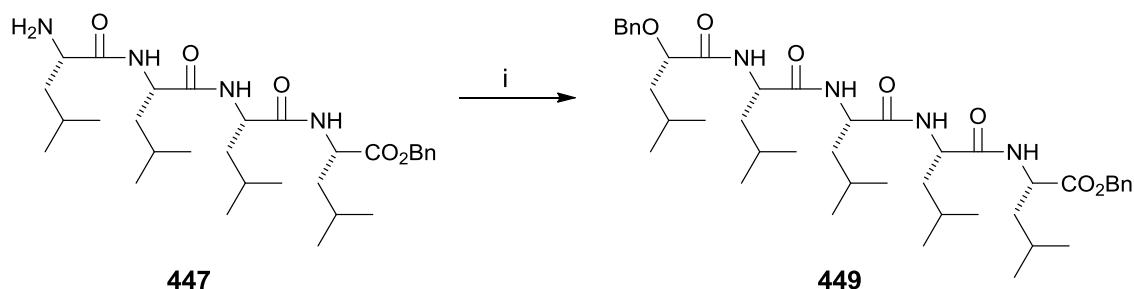
2.11.2. Coupling of protected hydroxy carboxylic acid to leucine tetramer

To allow for synthesis of the centre phosphorus unit the molecule requires a hydroxyl group to be inserted. Following on from the earlier synthesis of **419** (Scheme 107), it is possible to use NaH, a strong base, to initiate benzylation of the alcohol instead of the carboxylic acid.



Scheme 121 *Reagents and conditions:* (i) NaH, DMF 60 °C 1 h; (ii) BnBr (0 °C for addition) 60 °C 3 h.

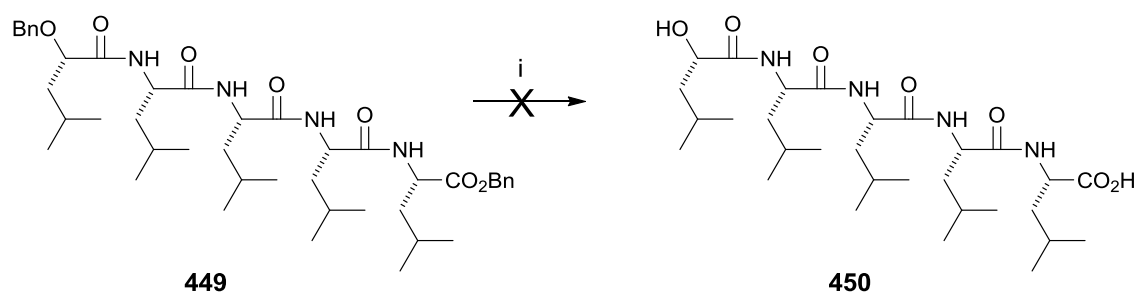
The synthesis of the benzyl protected alcohol **448** that will be coupled to the tetramer of leucine is discussed in **Scheme 121**. The reaction converted diacid **416** to compound **448**.



Scheme 122 *Reagents and conditions:* (i) **448**, DCC, DMAP, CH₂Cl₂.

Coupling of compound **448** to compound **447** was carried out using the same conditions for the stepwise chain extension (**Scheme 122**). The reaction afforded the desired dibenzyl protected pentamer product **449**.

2.11.3. Deprotection of “pentamer” of leucine



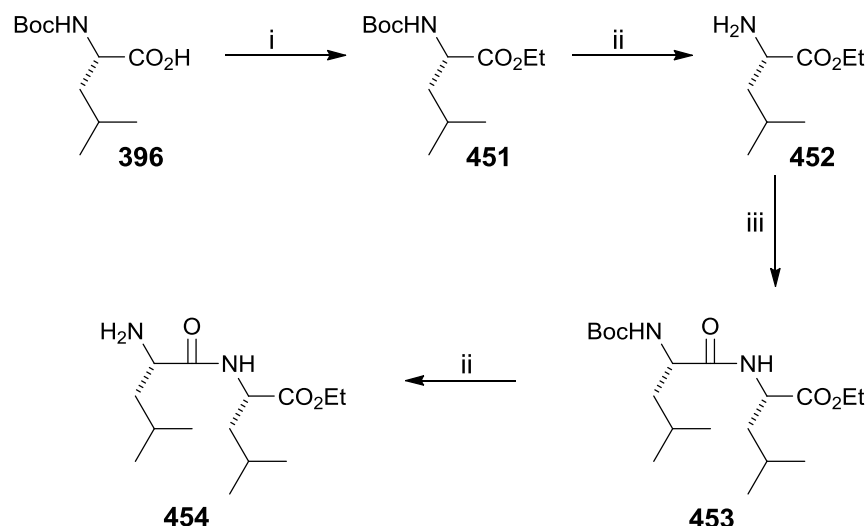
Scheme 123 Reagents and conditions: (i) Pd/C 5%, H₂, EtOAc.

The last step before addition of the phenyl phosphinate requires deprotection, which can be carried out by catalytic hydrogenation. Compound **449** was treated under hydrogenation conditions to form the fully deprotected “pentamer” **450** (Scheme 123). Unfortunately hydrogenation did not remove both of the protecting groups as planned; instead only one benzyl groups was removed, confirmed by mass spectroscopy. As both ends of “pentamer” could not be deprotected, the use of this “pentamer” had to be stopped and an alternate one synthesised.

2.11.4. Alternative method for tetramer synthesis

Whilst the stepwise elongation of the amino acid chain was suitable at first, the problems with deprotection required modifications to the synthesis. As all of tetramer **446** was used for the formation of “pentamer” **449** it was not possible to carry out a transesterification, therefore it was necessary to begin the synthesis again. This time it was decided to divide the tetramer component synthesis into two parts to reduce the number of steps.

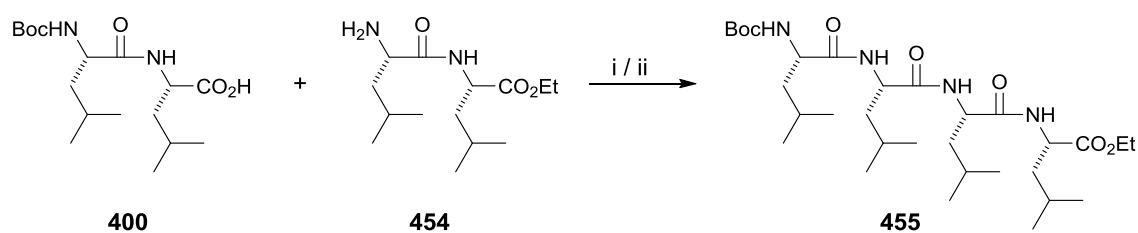
The synthesis of the first part is discussed in Scheme 124.



Scheme 124 *Reagents and conditions:* (i) EtOH, DCC, DMAP; (ii) TFA, CH₂Cl₂, 0 °C 30 min; (iii) Boc-(L)-Leu-OH, DCC, DMAP, CH₂Cl₂.

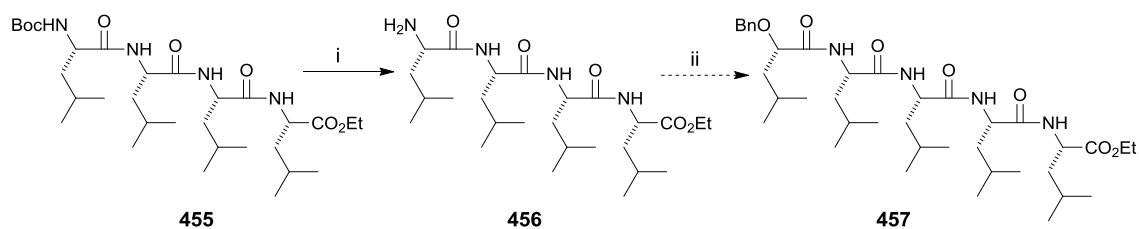
The synthesis began by protecting the carboxylic acid of Boc-(L)-Leu-OH **396** as an ethyl ester to give **451**. Deprotection of the Boc group of **451** gave **452** which was then coupled with Boc-(L)-Leu-OH **396** to give **454**, forming the first component.

The second part (**400**) to be combined with **454** to form the tetramer was already discussed in **Scheme 95**.



Scheme 125 *Reagents and conditions:* (i) DCC, DMAP, CH₂Cl₂; (ii) HBTU, Et₃N, THF.

Tetramer **455** (**Scheme 125**) is formed by coupling **400** and **454** using peptide coupling conditions to give the desired tetramer.



Scheme 126 *Reagents and conditions:* (i) TFA, CH₂Cl₂, 0 °C 30 min; (ii) **448**, DCC, DMAP, CH₂Cl₂.

The method for forming compound **457** is outlined in **Scheme 126**. As previously, formation of “pentamer” **457** requires removal of the Boc protecting group from **455**, followed by coupling with **448**. Due to time constraints the final step is pending, but once complete can be used to form the decamer compound.

2.12. Inhibition of cathepsin C

Dipeptidyl Peptidase I (DPP-I aka cathepsin C) is a cysteine dipeptidyl aminopeptidase that is expressed in the lysosomes of several tissues, the highest level being in the lung, macrophages, neutrophils, CD8⁺ T cells and mast cells. The classification of DPP-I as part of the lysosomal papain-type cysteine proteinase family (which also includes cathepsins B, H, K, L, O, and S) is based on its localization in the cell, acidic pH optimum for enzyme activity, and conserved amino acid sequence with respect to the NH₂-terminal and COOH-terminal regions that form the substrate-binding pocket of the enzyme.

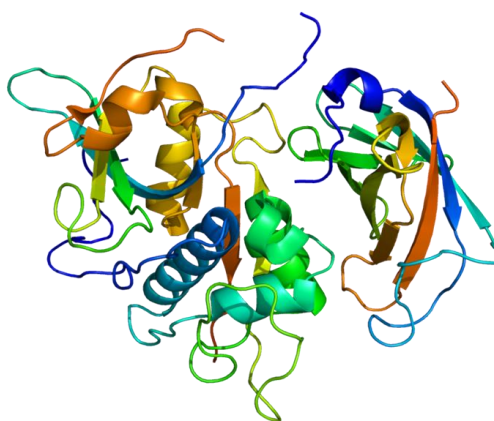


Figure 30 The crystal structure of human dipeptidyl peptidase I (cathepsin C) in complex with the inhibitor Gly-Phe-CHN₂.¹⁰¹

DPP-I is an oligomeric protein (**Figure 30**) with exclusive exopeptidase activity, which is different from the other cathepsins (B, H, K, L, O, and S) that are monomeric proteins with endopeptidase activity. As well as this significant difference, the overall amino acid sequence homology of DPP-I shows relatively little similarity with the other members of this group of proteinases. It is the only cysteine proteinase that has so far been shown to exist in polymorphonuclear leukocytes (PMNL).¹⁰¹ PMNL are a category of granulocytes specifically neutrophil granulocytes. These are white blood cells that have a lobed nucleus and granules in their cytoplasm.

Cathepsin C is thought to play an important role in intracellular protein degradation. The enzyme is responsible for the cleavage of two residues from the N- termini of proteins. It has a broad specificity and will continue cleaving residues until it finds a stop sequence, usually arginine or lysine in the N-terminal position (P2), proline on either side of the scissile bond (P1 / P1') or isoleucine (P1). The pro form contains the pro peptide and a catalytic region, which can be further processed into heavy/light chains that are linked by a disulfide bond.

It has been shown that cathepsin C activates several chymotrypsin-like serine proteases by removing an amino-terminal dipeptide. In addition, cathepsin C is involved in the similar cleavages in several other proteases allowing for their full activation. These include cathepsin G1 (CG), proteinase-3 (Pr-3), neutrophil elastase (NE), granzymes A, B, C and mast cell chymase and tryptase.

It is thought that serine proteases, and especially neutrophil elastase play a key role in inflammatory disease. This can be shown by the early onset of emphysema in patients deficient in plasma α 1AT, the main neutrophil elastase inhibitor in the lung. Similarly with cystic fibrosis, an excess of NE leads to inflammation and tissue destruction. Disturbance of the normal balance of enzymatic activity, which is in part affected by cathepsin C, may lead to many other pathological conditions, such as rheumatoid arthritis and osteoarthritis, cancer, neurological disorders, osteoporosis and lysosomal storage diseases.

For example, it has been shown that in vitro, NE affects the levels of $\text{TNF}\alpha$ ^{102,103} and $\text{TGF}\alpha$, and stimulates mucus secretion¹⁰². NE may potentiate the oncogenicity of PML-RAR α in acute promyelocytic leukaemia and in various other cancers.^{104; reviewed in 105)}

Another serine protease, mast cell tryptase, is thought to participate to asthma pathophysiology through its effect on bronchodilating peptides and protease activated

receptor-2 (PAR-2).^{106,107} CG and Pr-3 have distinct in vitro effects on cell signalling and cytokine and chemokine processing. CG modulates chemokine and reactive oxygen species release in murine PMN¹⁰⁸, and activates PAR-4.¹⁰⁹

Clearly then, inhibition of cathepsin C will result in a reduced activity within several clinically important serine protease targets, and could possibly have greater efficacy than simply blocking individual serine proteases. This is of specific importance in complex inflammatory diseases such as chronic obstructive pulmonary disease (COPD).

However, of the selective and potent cathepsin C inhibitors that have been found, most have a poor metabolic stability and are cytotoxic. A brief summary of the known inhibitors of cathepsin C are given below (**Figure 31**, **Table 40** and **Table 41**). These include irreversible inhibitors (**Figure 31**), non-phosphorus containing pseudopeptides (**Table 40**) and phosphorus containing reversible inhibitors (**Table 41**). In particular, one of the potent, reversible inhibitors of DPPI is Leu-Leu-OMe **458**. Based on this observation, we reasoned that Leu(P)-Leu-OMe (compound **426**) and analogue **431** should also be a potent reversible inhibitor.

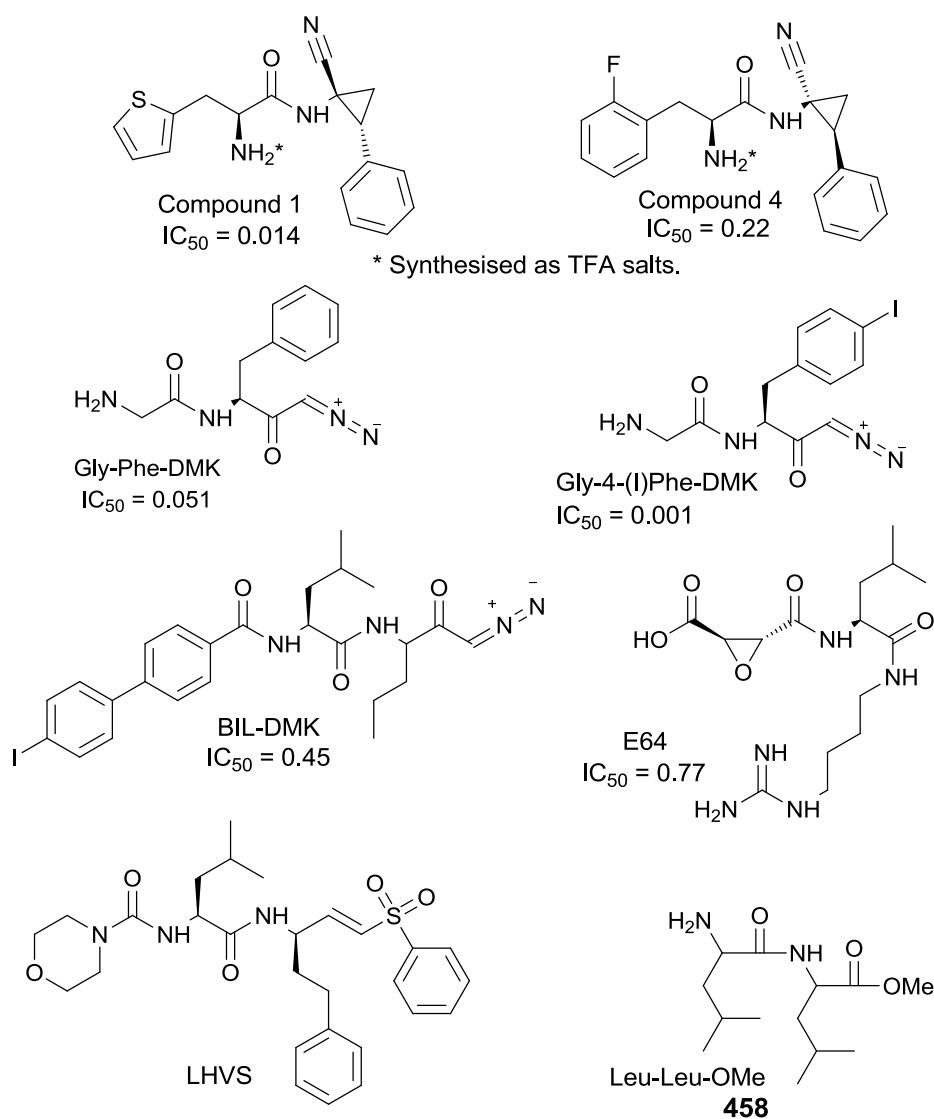


Figure 31 Various inhibitors of cathepsin C currently known. The activity ranges from >10 to 0.001 μ M with Gly-4-(I)Phe-DMK.¹⁰¹

| <p>Gly-Δ^EPhe-Gly-Phe-pNA</p> | | <p>Gly-Gly-Δ^EPhe-Phe-pNA</p> | |
|---|-------------------|---|-------------------|
| Inhibitor | K _M mM | Inhibitor | K _M mM |
| Gly- Δ^E -Phe-Gly-Phe-pNA | 3.2 | Gly-Gly- Δ^E -Phe-Phe-pNA | 4.3 |
| Gly- Δ^Z -Phe-Gly-Phe-pNA | 7.8 | Gly-Gly- Δ^Z -Phe-Phe-pNA | 7.8 |
| Gly-Phe-Gly-Phe-pNA | 22.5 | Gly-Gly-Phe-Phe-pNA | Insoluble |
| Gly-Phe-pNA | 0.23 | | |

Table 40 Various inhibitors of cathepsin C synthesised by Kafarski group.¹¹⁰

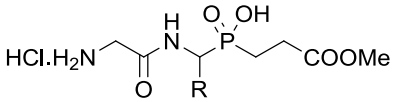
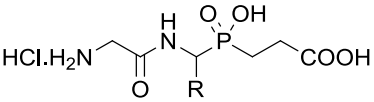
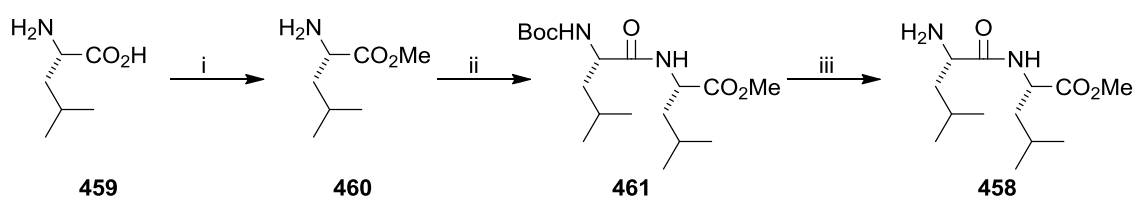
|  | |  | |
|---|-------------------|--|-------------------|
| R | K _i mM | R | K _i mM |
| CH ₂ CH(CH ₃) ₂ | 0.040 | CH ₂ CH(CH ₃) ₂ | 0.039 |
| Ph | 0.188 | Ph | 0.176 |
| CH ₂ Ph | 0.514 | CH ₂ Ph | 0.429 |
| CH ₂ CH ₂ Ph | 0.312 | CH ₂ CH ₂ Ph | 0.187 |

Table 41 Various cathepsin C inhibitors of synthesised by Kafarski group.^{57e}



Scheme 127 Reagents and conditions: (i) AcCl, MeOH, 0 °C; (ii) Boc-(L)-Leu-OH, DCC, DMAP, CH₂Cl₂; (iii) TFA, CH₂Cl₂, 0 °C 30 min.

The synthesis of the target compound began with L-leucine, which was transformed into its methyl ester by treatment with acetyl chloride in methanol to give **460**. Coupling of **460** with Boc-(L)-Leu-OH using DCC and DMAP as coupling reagents gave the corresponding diamino acid **461**. The final step towards the target compound involved deprotection of the amino acid using TFA to give the corresponding amine product **458**.

Both compounds **426** and **431** were tested in an enzyme hydrolysis assay. Action of the enzyme on Ala-Ala-*p*NA releases *para*-nitroaniline. The rate of the enzyme reaction was measured as the increase in the absorption of the reaction mixture at $\lambda = 405$ nm. Action of the enzyme on Ala-Ala-*p*NA both in the presence of the test compounds and reference (blank) were measure but no difference was found. Therefore, we concluded that neither **426** nor **431** are inhibitors of cathepsin C.

3. Chapter Three: Experimental Section

3.1. General methods and instrumentation

Commercially available reagents were used as received without additional purification. All solvents were of reagent grade. Petroleum ether refers to the fraction of petroleum spirit boiling in the range of 60 to 80 °C. Where stated, mixtures of solvents are referred to as percentage volume to volume (v/v) ratios.

Products were typically purified by column chromatography using Merck 9385 silica gel 60 (40-63 μm). Analytical thin layer chromatography (TLC) was conducted on Merck silica gel 60 F₂₅₄ glass backed plates. Visualisation of the reaction components was accomplished by illumination under short wavelength (254 nm) ultraviolet light or using basic potassium permanganate (KMnO₄) stain.

Microanalyses were performed at the London Metropolitan University. All melting point (mp) values were determined on a Gallenkemp melting point apparatus and are stated uncorrected.

Routine infrared (IR) spectra were recorded on a Perkin-Elmer FTIR spectrometer using sodium chloride plates or made using KBr discs. Spectra are reported in wavenumbers (cm⁻¹) with the following abbreviations; strong (s), medium (m), weak (w) and broad (br).

Proton Nuclear Magnetic Resonance (¹H NMR) spectra were recorded using Bruker AMX400 (400 MHz) and JEOL ECA-600 (600 MHz) spectrometers as stated. Chemical shifts are reported in parts per million (δ , ppm) with internal reference to tetramethyl silane. Coupling constants (*J*) are expressed in Hertz (Hz) and spectra are reported with the following abbreviations; singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint.), multiplet (m) and broad (br).

Carbon Nuclear Magnetic Resonance (¹³C NMR) were performed on the same instruments operating at 101 and 150 MHz respectively. Phosphorus Nuclear Magnetic

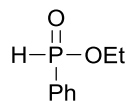
Resonance (^{31}P NMR) were performed on the same instruments operating at 162 and 243 MHz respectively. Deuterated chloroform-d (CDCl_3) was used as the NMR solvent unless otherwise stated.

^1H - ^1H connectivities were determined using DQF COSY; ^1H - ^{13}C $^1J_{\text{CH}}$ connectivities by HMQC and $^2J_{\text{CH}}$ and $^3J_{\text{CH}}$ using HMBC; ^1H - ^{31}P $^2J_{\text{PH}}$, $^3J_{\text{PH}}$ and $^4J_{\text{PH}}$ connectivities were determined using HOESY experiments.

Routine mass spectra were run on a Micromass Quattro Ultima spectrometer in the electrospray ionisation (ESI), positive (+ve) or negative (-ve) mode as stated. High resolution mass spectrometry was performed at the National Mass Spectrometry Centre Swansea using MAT95 or MAT900 in the electrospray ionisation (ESI) mode.

3.2. Synthetic procedures

Ethyl phenylphosphinate **320**



Pyridine (12.8 ml, 158 mmol) was slowly added to a vigorously stirred solution of ethyl chloroformate (16 ml, 100 mmol) and compound **322** (22.4 g 157 mmol) in chloroform (350 ml) at room temperature. (Caution! Exothermic Reaction; Rapid Effervescence.) Once the effervescence had stopped, the solution was stirred in a 40 °C water bath for 15 min. The solution was poured into 0.1M HCl (aq) (60 ml) and the organic layer was extracted. The organic extracts were washed with water (60 ml) and then dried over Na₂SO₄. The solvent was removed *in vacuo* to give the title compound as a colourless oil (30 g, quantitative yield).

$\nu_{\text{max}}/\text{cm}^{-1}$ 1000-1300 CO, 1653-2102 P=O, 2843-3600 CH

δ_{P} (162 MHz, CDCl₃) 24.54

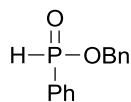
δ_{H} (400 MHz, CDCl₃) 1.33 (3H, t, J 7.1, OCH₂CH₃), 4.04-4.20 (2H, m, OCH₂CH₃), 7.44-7.49 (2H, m, Ar H), 7.54 (1H, d, J_{P} 563.0, PH), 7.53-7.58 (1H, m, Ar H), 7.71-7.78 (2H, m, Ar H).

δ_{C} (101 MHz, CDCl₃) 16.74 (d, J_{P} 6.5, OCH₂CH₃), 62.67 (d, J_{P} 6.4, OCH₂CH₃), 129.20 (d, J_{P} 13.9, Ar CH), 131.33 (d, J_{P} 11.9, Ar CH), 131.52 (d, J_{P} 123.1, Ar CH), 133.59 (d, J_{P} 3.0, Ar CH).

LRMS m/z (ESI) 363.1 [M₂+H]⁺, 193 [M+Na]⁺, 171 [M+H]⁺.

HRMS (ESI) calcd. for C₈H₁₁O₂PNa 193.0394, found 193.0391 [M+Na]⁺.

Benzyl phenylphosphinate **321**



Pyridine (5.6 ml, 70 mmol) was slowly added to a vigorously stirred solution of benzyl chloroformate (8.3 ml, 58 mmol) and compound **322** (10 g, 7 mmol) in dichloromethane (150 ml) at room temperature. (Caution! Exothermic process; Rapid Effervescence). Once the effervescence had stopped, the solution was left stirring in a water bath at 40 °C for 15 min. The solution was poured into 0.1M HCl (aq) (60 ml) and the organic layer was extracted. The organic extracts were washed with water (80 ml), and then dried over Na₂SO₄. The solvent was removed *in vacuo* to give the title compound (12 g, quant. yield) as a colourless oil.

$\nu_{\text{max}}/\text{cm}^{-1}$ 1035-2526 P=O, 2833-3305 CH

δ_{P} (162 MHz, CDCl₃) 24.99

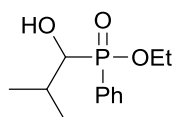
δ_{H} (400 MHz, CDCl₃) 5.12 (1H, q, J 12.0, OCH₂Ar), 5.13 (1H, q, J 12.0, OCH₂Ar), 7.28-7.48 (5H, m, Ar H), 7.50-7.52 (2H, m, Ar H), 7.58-7.61 (1H, m, Ar H), 7.65 (1H, d, J_{P} 566.0, PH), 7.77-7.83 (2H, m, Ar H).

δ_{C} (101 MHz, CDCl₃) 65.55 (OCH₂Ar), 128.16 (d, J_{P} 135.2, Ar C), 128.20 (d, J_{P} 140.0, Ar C), 129.07 (d, J_{P} 17.4, Ar CH), 129.19 (d, J_{P} 18.0, Ar CH), 131.13 (d, J_{P} 12.1, Ar CH), 131.45 (d, J_{P} 12.0, Ar CH), 133.22 (d, J_{P} 2.9, Ar CH), 133.70 (d, J_{P} 3.0, Ar CH).

LRMS m/z (ESI) 487.1 [M₂+Na]⁺, 339.1 [M+Na]⁺, 233 [M+H]⁺.

HRMS (ESI) calcd. for C₁₃H₁₃O₂PNa 255.0550, found 255.0550 [M+Na]⁺.

Ethyl (1-hydroxy-2-methylpropyl)(phenyl)phosphinate **337**¹¹¹



Compound **320** (20 g, 118 mmol) was mixed with isobutyraldehyde (11.8 ml, 129 mmol). KF (60 g, 689 mmol) was added and the mixture was left to stir overnight. dichloromethane (80 ml) was added and the reaction was allowed to stir for 30 min. KF was filtered off through celite and the solvent was removed *in vacuo*. The residue was subjected to column chromatography (EtOAc : Pet. Ether 3:1 - 1:1) to give the title compound as a colourless oil (28.6 g, 118 mmol, quant. yield), a mixture of two diastereomers.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3263, 2856-2963, 1714, 1592, 1468, 1438, 1390, 1366, 1338, 1236, 1190, 1118, 1097, 1020.

δ_{P} (162 MHz, CDCl_3) 40.22, 40.24.

δ_{H} (400 MHz, CDCl_3) 1.00 (3H, d, J 6.7, CH_3), 1.01 (3H, d, J 6.7, CH_3), 1.03 (3H, d, J 6.6, CH_3), 1.06 (3H, d, J 6.7, CH_3), 1.32 (3H, t, J 7.1, OCH_2CH_3), 1.33 (3H, t, J 7.1, OCH_2CH_3), 1.90-1.98 (1H, m, CHMe_2), 2.00-2.12 (1H, m, CHMe), 3.75 (1H, dd, J 3.7, 4.7, PCH), 3.77 (1H, dd, J 4.6, 5.8, PCH), 3.87-4.03 (2H, m, OCH_2CH_3), 4.08-4.21 (2H, m, OCH_2CH_3), 7.46-7.51 (4H, m, Ar H), 7.55-7.60 (2H, m, Ar H), 7.81-7.87 (4H, m, Ar H).

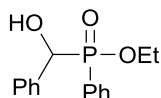
δ_{C} (101 MHz, CDCl_3) 16.53 (d, J_{P} 6.0, OCH_2CH_3), 16.60 (d, J_{P} 6.0, OCH_2CH_3), 17.32 (d, J_{P} 6.0, CH_3), 17.97 (d, J_{P} 7.3, CH_3), 20.17 (d, J_{P} 8.2, CH_3), 20.28 (d, J_{P} 10.2, CH_3), 29.40 (d, J_{P} 3.9, CHMe_2), 29.59 (d, J_{P} 3.0, CHMe_2), 61.15 (d, J_{P} 7.2, OCH_2CH_3), 61.28 (d, J_{P} 7.0, OCH_2CH_3), 74.44 (d, J_{P} 113.8, PCH), 75.13 (d, J_{P} 110.1, PCH), 128.47 (d, J_{P} 12.1, Ar CH), 128.52 (d, J_{P} 12.1, Ar CH), 128.71 (d, J_{P} 118.6, Ar C), 129.36 (d, J_{P}

118.9, Ar C), 132.26 (d, J_P 9.5, Ar CH), 132.41 (d, J_P 9.5, Ar CH), 132.54 (d, J_P 2.7, 2 x Ar CH).

LRMS m/z (ESI) 265.2 $[M+Na]^+$, 243.2 $[M+H]^+$.

HRMS (ESI) calcd. for $C_{12}H_{20}O_3P$ 243.1145, found 243.1142 $[M+H]^+$.

Ethyl (hydroxy(phenyl)methyl)(phenyl)phosphinate **338**



Compound **320** (10 g, 60 mmol) was mixed with benzaldehyde (6 ml, 60 mmol). KF (20 g) was added and the mixture was left to stir overnight. dichloromethane (80ml) was added and the reaction was allowed to stir for 30 min. KF was filtered off using celite and the solvent was removed *in vacuo* to give the title compound as a white solid, a mixture of diastereomers. (16 g, 97% crude yield). Recrystallisation of the solid diastereomers using EtOAc afforded two products.

Diastereomer 1 (**338a**) (1.0 g, 6% yield):

mp 119-121 °C.

$\nu_{\max}/\text{cm}^{-1}$ 1000-1491 P=O, 2800-3058 CH, 3264 OH

δ_P (243 MHz, CDCl_3) 39.34

δ_H (600 MHz, CDCl_3) 1.32 (3H, t, J 7.1, CH_3), 3.95-4.01 (1H, m, OCH_2), 4.11-4.17 (1H, m, OCH_2CH_3), 4.28 (1H, br s, OH), 5.17 (1H, d, J 11.0, PCH), 7.20-7.22 (5H, m, Ar H), 7.30-7.32 (2H, m, Ar H), 7.42-7.44 (2H, m, Ar H), 7.46-7.49 (1H, m, Ar H).

δ_C (151 MHz, CDCl_3) 16.62 (d, J_P 6.0, OCH_2CH_3), 61.95 (d, J_P 7.2, OCH_2CH_3), 73.34 (d, J_P 110.7, PCH), 127.04 (d, J_P 122.83, Ar C), 127.05 (d, J_P 5.0, Ar CH), 127.86 (d, J_P

3.3, Ar CH), 128.03 (d, J_P 2.7, Ar CH), 128.13 (d, J_P 12.4, Ar CH), 132.61 (d, J_P 2.8, Ar CH), 132.97 (d, J_P 9.2, Ar CH), 136.38 (d, J_P 1.1, Ar C).

LRMS m/z (ESI) 277.0 $[M+H]^+$.

Diastereomer 2 (**338b**) (1.2 g, 7% yield):

mp 101-104 °C.

$\nu_{\max}/\text{cm}^{-1}$ 1000-1500 P=O, 2850-3059 CH, 3263 OH

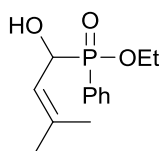
δ_P (243 MHz, CDCl_3) 37.98.

δ_H (600 MHz, CDCl_3) 1.26 (3H, t, J 7.1, CH_3), 4.03-4.09 (1H, m, OCH_2), 4.11-4.16 (1H, m, OCH_2CH_3), 4.3 (1H, br s, OH), 5.10 (1H, d, J 7.5, PCH), 7.20-7.23 (5H, m, Ar H), 7.29-7.31 (2H, m, Ar H), 7.51-7.53 (2H, m, Ar H), 7.62-7.63 (1H, m, Ar H).

δ_C (151 MHz, CDCl_3) 16.58 (d, J_P 5.8, OCH_2CH_3), 61.78 (d, J_P 6.9, OCH_2CH_3), 73.68 (d, J_P 110.2, PCH), 127.38 (d, J_P 5.2, Ar CH), 127.80 (d, J_P 3.2, Ar CH), 127.98 (d, J_P 2.7, Ar CH), 128.11 (d, J_P 124.2, Ar C), 128.32 (d, J_P 12.4, Ar CH), 132.59 (d, J_P 2.9, Ar CH), 133.00 (d, J_P 9.2, Ar CH), 136.17 (d, J_P 1.6, Ar C).

LRMS m/z (ESI) 277.0 $[M+H]^+$.

Ethyl (1-hydroxy-3-methylbut-2-en-1-yl)(phenyl)phosphinate **340**



Compound **320** (15 g, 88 mmol) was mixed with 3-methyl-2-butenal (8.5 ml, 88 mmol). KF (30 g) was added and the mixture was stirred until it was completely solid. dichloromethane (80 ml) was added and the reaction was allowed to stir for 30 min. KF was filtered off through celite and the solvent was removed *in vacuo*. Recrystallisation

using EtOAc gave the title compound as colourless crystals (First crop 6.0 g, 27%).

Diastereomer 1 (**340a**) (Crystalline):

mp 113-115 °C.

Found: C, 61.40; H, 7.60; C₁₃H₁₉O₃P requires C, 61.41; H, 7.53;

$\nu_{\max}/\text{cm}^{-1}$ 3273 (OH), 2950-2848 (CH), 1440, 1226, 1211, 1163, 1121, 1051 (P=O)

δ_{P} (162 MHz, CDCl₃) 38.77

δ_{H} (400 MHz, CDCl₃) 1.35 (3H, t, J 7.0, OCH₂CH₃), 1.47 (3H, d, J 4.0, CH₃), 1.69 (3H, d, J 4, CH₃), 3.90-3.93 (1H, t, J_{P} 6.0, OH), 4.00-4.11 (1H, m, OCH₂CH₃), 4.17-4.26 (1H, m, OCH₂CH₃), 4.73 (1H, ddd, J 4.0, 6.0, J_{P} 10.0, PCH), 5.13-5.16 (1H, m, CH=CMe₂), 7.44-7.58 (3H, m, Ar H), 7.75-7.80 (2H, m, Ar H).

δ_{C} (101 MHz, CDCl₃) 17.0 (d, J_{P} 5.0, OCH₂CH₃), 18.9 (d, J_{P} 3.0, CH₃), 26.3 (d, J_{P} 3.0, CH₃), 62.04 (d, J_{P} 7.0, OCH₂CH₃), 68.8 (d, J_{P} 116.0, PCH), 120.13 (=CMe₂), 128.9 (d, J_{P} 121.0, Ar C), 128.7 (d, J_{P} 12.0, Ar CH), 132.8 (d, J_{P} 3.0, Ar CH), 133.1 (d, J_{P} 10.0, Ar CH), 139.1 (d, J_{P} 13.0, CH=CMe₂), 139.43 (=CMe₂).

LRMS m/z (ESI) 254 [M+H]⁺.

HRMS (ESI) calcd. for C₁₃H₁₉O₃PNa 277.0964, found 277.0968 [M+Na]⁺.

Diastereomer 2 (**340b**) (Oil):

$\nu_{\max}/\text{cm}^{-1}$ 3275 (OH), 2954-2854 (CH), 1450, 1377, 1226, 1214, 1160, 1055 (P=O)

δ_{P} (162 MHz, CDCl₃) 38.25

δ_{H} (400 MHz, CDCl₃) 1.35 (3H, t, J 6.5, OCH₂CH₃), 1.50 (3H, d, J 4.0, CH₃), 1.76 (3H, d, J 4.0, CH₃), 3.23 (1H, br s, OH), 4.03-4.13 (1H, m, OCH₂CH₃), 4.16-4.25 (1H, m,

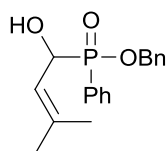
OCH₂CH₃), 4.72 (1H, dd, *J* 3.5, *J*_P 9.60, PCH), 5.24-5.28 (1H, m, CH=CMe₂), 7.47-7.50 (2H, m, Ar H), 7.55-7.57 (1H, m, Ar H), 7.80-7.85 (2H, m, Ar H).

δ_C (101 MHz, CDCl₃) 16.59 (d, *J*_P 5.8, OCH₂CH₃), 18.54 (d, *J*_P 1.6, CH₃), 26.01 (d, *J*_P 2.1, CH₃), 61.66 (d, *J*_P 7.1, OCH₂CH₃), 68.42 (d, *J*_P 115.3, PCH), 119.04 (=CMe₂), 128.74 (d, *J*_P 121.7, Ar C), 128.32 (d, *J*_P 12.0, Ar CH), 128.39 (d, *J*_P 12.0, Ar CH), 132.43 (d, *J*_P 9.3, Ar CH), 139.69 (d, *J*_P 11.3, CH=CMe₂), 139.81 (=CMe₂).

LRMS *m/z* (ESI) 254 [M+H]⁺.

HRMS (ESI) calcd. for C₁₃H₁₉O₃PNa 277.0964, found 277.0966 [M+Na]⁺.

Benzyl (1-hydroxy-3-methylbut-2-en-1-yl)(phenyl)phosphinate **341**



Compound **320** (12.85 g, 55 mmol) was mixed with 3-methyl-2-butenal (5.4 ml, 56 mmol). KF (24 g) was added and the mixture was left to stir overnight. dichloromethane (80 ml) was added and the reaction was allowed to stir for 30 min. KF was filtered off through celite and the solvent was removed *in vacuo*. The product was a mixture of two diastereomers, both solids. Recrystallisation using diethyl ether gave the title compound as white crystals.

Diastereomer 1 (**341a**) (2.33 g, 13% yield):

mp 120-125 °C.

ν_{max}/cm⁻¹ 1025-1669 P=O, 2853-2924 CH, 3233 OH

δ_P (162 MHz, CDCl₃) 38.86

δ_{H} (400 MHz, CDCl_3) 1.50 (3H, d, J 4.0, CH_3), 1.75 (3H, d, J 4.0, CH_3), 2.96 (1H, dd, J 4.7, 6.0, OH), 4.77 (1H, dt, J 4.0, J_{P} 9.5, PCH), 5.00 (1H, dd, J 6.7, 11.8, OCH_2Ph), 5.20 (1H, dd, J 6.7, 11.8, OCH_2Ph), 5.25-5.29 (1H, m, $\text{CH}=\text{CMe}_2$), 5.25-5.29 (1H, m, OCH_2Ph), 7.33-7.39 (5H, m, Ar H), 7.46-7.51 (2H, m, Ar H), 7.57-7.61 (1H, m, Ar H), 7.82-7.87 (2H, m, Ar H).

δ_{C} (101 MHz, CDCl_3) 18.97 (d, J_{P} 1.7, CH_3), 26.31 (d, J_{P} 2.1, CH_3), 67.17 (d, J_{P} 7.0, CH_2Ar), 69.03 (d, J_{P} 115.0, PCH), 120.01 (d, J_{P} 2.6, Ar C), 128.74 (d, J_{P} 12.7, Ar CH), 128.46 (d, J_{P} 39.7, Ar CH), 128.58 (d, J_{P} 120.8, Ar C), 128.80 (d, J_{P} 23.2, Ar CH), 128.87 (d, J_{P} 10.5, Ar CH), 132.97 (d, J_{P} 2.6, Ar CH), 133.11 (d, J_{P} 9.3, Ar CH).

LRMS m/z (ESI) 339.1 $[\text{M}+\text{Na}]^+$.

HRMS (ESI) calcd. for $\text{C}_{18}\text{H}_{21}\text{O}_3\text{PNa}$ 339.1126, found 339.1116 $[\text{M}+\text{Na}]^+$.

Diastereomer 2 (**341b**) (1.8 g, 10% yield):

mp 105-110 °C.

$\nu_{\text{max}}/\text{cm}^{-1}$ 1083-1668 $\text{P}=\text{O}$, 2853-2922 CH, 3283 OH

δ_{P} (162 MHz, CDCl_3) 39.53

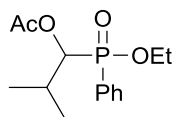
δ_{H} (400 MHz, CDCl_3) 1.47 (3H, d, J 4.0, CH_3), 1.70 (3H, d, J 4.0, CH_3), 2.83 (1H, dd, J 5.5, 6.1, OH), 4.78 (1H, dt, J 4.0, J_{P} 9.6, PCH), 5.00 (1H, dd, J 6.8, 11.8, OCH_2Ph), 5.14-5.18 (1H, m $\text{CH}=\text{CMe}_2$), 5.22 (1H, dd, J 6.7, 11.8, OCH_2Ph), 7.32-7.40 (5H, m, Ar H), 7.45-7.50 (2H, m, Ar H), 7.56-7.60 (1H, m, Ar H), 7.78-7.83 (2H, m, Ar H).

δ_{C} (101 MHz, CDCl_3) 19.00 (d, J_{P} 1.6, CH_3), 26.42 (d, J_{P} 2.0, CH_3), 67.09 (d, J_{P} 6.7, CH_2Ar), 68.95 (d, J_{P} 115.0, PCH), 119.22 (d, J_{P} 2.8, Ar C), 128.47 (d, J_{P} 41.7, Ar CH), 128.74 (d, J_{P} 8.0, Ar CH), 128.83 (d, J_{P} 121.7, Ar C), 128.86 (d, J_{P} 14.1, Ar CH), 128.94 (d, J_{P} 1.9, Ar CH), 132.87 (d, J_{P} 8.4, Ar CH), 133.03 (d, J_{P} 2.8, Ar CH).

LRMS m/z (ESI) 339.1 $[M+Na]^+$.

HRMS (ESI) calcd. for $C_{18}H_{21}O_3PNa$ 339.1126, found 339.1123 $[M+Na]^+$.

1-(ethoxy(phenyl)phosphoryl)-2-methylpropyl acetate **342¹¹¹**



Acetic anhydride (0.44 ml, 5 mmol), was added to a stirred solution of compound **337** (1.01 g, 4 mmol) in dichloromethane (20 ml) maintained at 0 °C. The solution was allowed to stir for 1 h and monitored by TLC. On completion of the reaction the solvent was removed *in vacuo*. The reaction mixture diluted with dichloromethane, poured into water (50 ml) and the organic layer was extracted with dichloromethane (2 x 25 ml). The combined organic extracts were washed with saturated $NaHCO_3$, followed by brine and then dried over Na_2SO_4 and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 5:1 - 2:1) to give the title compound (1.18 g, 4 mmol, quant. yield) a mixture of diastereomers as a colourless oil.

ν_{max}/cm^{-1} 3264, 2865-2962, 1747, 1592, 1468, 1439, 1390, 136,7 1191, 1119, 1025.

δ_P (162 MHz, $CDCl_3$) 40.30, 40.32.

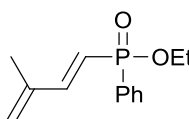
δ_H (400 MHz, $CDCl_3$) 1.00 (6H, d, J 6.7, $\underline{CH_3}$), 1.02 (3H, d, J 6.2, $\underline{CH_3}$), 1.06 (3H, d, J 6.7, $\underline{CH_3}$), 1.32 (3H, t, J 7.1, $OCH_2\underline{CH_3}$), 1.33 (3H, t, J 7.1, $OCH_2\underline{CH_3}$), 1.45 (6H, s, $CO\underline{CH_3}$), 1.87-1.99 (1H, m, $\underline{CH}Me_2$), 2.02-2.14 (1H, m, $\underline{CH}Me_2$), 3.74-3.78 (2H, m, 2 x $P\underline{CH}$), 3.89-4.03 (2H, m, $O\underline{CH_2}CH_3$), 4.10-4.21 (2H, m, $O\underline{CH_2}CH_3$), 7.46-7.51 (4H, m, Ar H), 7.55-7.59 (2H, m, Ar H), 7.82-7.86 (4H, m, Ar H).

δ_C (101 MHz, $CDCl_3$) 16.53 (d, J_P 5.9, OCH_2CH_3), 16.56 (d, J_P 5.8, OCH_2CH_3), 17.32 (d, J_P 5.9, CH_3), 17.96 (d, J_P 7.4, CH_3), 20.17 (d, J_P 9.9, CH_3), 20.27 (d, J_P 10.3, CH_3), 29.40 (d, J_P 4.0, CH_3), 29.59 (d, J_P 2.9, CH_3), 61.19 (d, J_P 7.2, OCH_2CH_3), 61.31 (d, J_P

7.0, OCH₂CH₃), 74.99 (d, J_P 113.7, PCH), 75.68 (d, J_P 110.0, PCH), 128.48 (d, J_P 12.1, Ar CH), 128.59 (d, J_P 12.1, Ar CH), 129.28 (d, J_P 119.1, Ar C), 129.32 (d, J_P 119.1, Ar C), 132.26 (d, J_P 9.5, Ar CH), 132.39 (d, J_P 9.7, Ar CH), 132.46 (d, J_P 3.0, Ar CH), 132.55 (d, J_P 2.7, Ar CH), 149.93 (CCO₂), 150.04 (CCO₂).

LRMS m/z (ESI) 285.3 [M+H]⁺, 243.2 [M-Ac+H]⁺.

(Z)-ethyl (3-methylbuta-1,3-dien-1-yl)(phenyl)phosphinate 344



Acetic anhydride (0.4 ml, 4 mmol) followed by sulphuric acid (0.02 ml, 0.4 mmol) were added to a stirred solution of compound **340a** (1.012 g, 4 mmol) in dichloromethane (150 ml) maintained at 0 °C. On completion of the reaction the reaction mixture was poured into saturated NaHCO₃ (50 ml) and the organic layer was separated. The organic phase was washed with NaHCO₃ (2 x 25 ml). The combined organic extracts were washed with brine and then dried over Na₂SO₄, stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 4:1 - 1:1) to give *the title compound* (0.9 g, 3.8 mmol, 95% yield) as a colourless oil.

$\nu_{\max}/\text{cm}^{-1}$ 2860-2957 1641 1553 1432 1388 1369 1277 1198 1133 1119

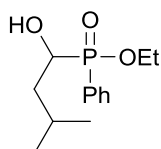
δ_P (162 MHz, CDCl₃) 31.49

δ_H (400 MHz, CDCl₃) 1.27 (3H, t, J 7.1, OCH₂CH₃), 1.79 (3H, s, CH₃), 3.87-3.97 (1H, m, OCH₂CH₃), 3.98-4.10 (1H, m, OCH₂CH₃), 5.23 (2H, s, CH₂), 5.84 (1H, dd, J 17.2, J_P 20.6, CH=CHMe₂), 7.09 (1H, dd, J 17.3, J_P 20.2, CH=CHMe₂), 7.38-7.43 (2H, m, Ar H), 7.45-7.50 (1H, m, Ar H), 7.71-7.77 (2H, m, Ar H).

δ_C (101 MHz, $CDCl_3$) 16.55 (d, J_P 6.6, OCH_2CH_3), 17.84 (CH_3), 60.75 (d, J_P 5.7, OCH_2CH_3), 118.30 (d, J_P 139.4, C=), 123.67 (CH_2), 127.81 (d, J_P 120.2, Ar C), 128.56 (d, J_P 12.9, Ar CH), 131.46 (d, J_P 10.0, Ar CH), 132.12 (d, J_P 2.8, Ar CH), 150.18 (d, J_P 5.3, =C).

LRMS m/z (ESI) 237.2 $[M+H]^+$

Ethyl (1-hydroxy-3-methylbutyl)(phenyl)phosphinate **347**



A mixture of compound **340a** (10.28 g, 40 mmol) and 10% Pd/C (2 g, 20% wt) in EtOAc (200 ml) was stirred under a hydrogen atmosphere overnight. The catalyst was filtered off using celite and the solvent was removed *in vacuo* to give the *title compound* as a white solid (10.36 g, 40 mmol, quant. yield).

mp 96-98 °C.

Found: C, 60.98; H, 8.10. $C_{13}H_{21}O_3P$ requires C, 60.93; H, 8.26.

ν_{max}/cm^{-1} 3259 (OH), 3062-2869 (CH), 1592, 1469, 1439, 1389, 1210 (P=O)

δ_P (162 MHz, $CDCl_3$) 40.49.

δ_H (400 MHz, $CDCl_3$) 0.88 (3H, d, J 6.6, $\underline{CH_3}$), 0.93 (3H, d, J 6.7, $\underline{CH_3}$), 1.35 (3H, t, J 7.1, $OCH_2\underline{CH_3}$), 1.40-1.45 (1H, m, $\underline{CH_2}CHMe_2$), 1.44-1.60 (1H, m, $\underline{CH_2}CHMe_2$), 1.85-1.93 (1H, m, $\underline{CH}Me_2$), 2.49 (1H, m, \underline{OH}), 3.98-4.08 (2H, m, $P\underline{CH}$ and $OCH_2\underline{CH_3}$), 4.08-4.26 (1H, m, $OCH_2\underline{CH_3}$), 7.43-7.73 (3H, m, Ar H), 7.75-7.85 (2H, m, Ar H).

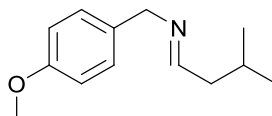
δ_C (101 MHz, $CDCl_3$) 15.6 (d, J_P 5.0, OCH_2CH_3), 20.0 (CH_3), 22.1 ($CHMe_2$), 22.53 (CH_3), 38.4 (CH_2), 60.4 (d, J_P 7.0, OCH_2CH_3), 67.3 (d, J_P 114.0, PCH), 127.3 (d, J_P

119.0, Ar C), 127.5 (d, J_p 12.0, Ar CH), 131.6 (d, J_p 9.0, Ar CH), 132.8 (d, J_p 3.0, Ar CH).

LRMS m/z (ESI) 257 $[M+H]^+$.

HRMS (ESI) calcd. for $C_{13}H_{21}O_3PNa$ 279.1121, found 279.1125 $[M+Na]^+$.

(4-Methoxy-benzyl)-[3-methyl-but-ylidene]-amine 348



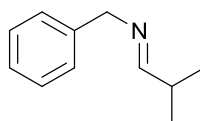
Isovaleraldehyde (6.4 ml, 60 mmol), and *p*-methoxy benzylamine (7.8 ml, 60 mmol), were added to toluene (100 ml) and heated under reflux overnight, using Dean-Stark apparatus. Once the reaction was complete the solvent was removed *in vacuo* to give the title compound (12 g, quant. yield) as a yellow oil which was used immediately in the next step without further purification.

δ_H (400 MHz, $CDCl_3$) 0.94 (3H, d, J 7.0, \underline{CH}_3), 0.98 (3H, d, J 7.0, \underline{CH}_3), 1.84 (1H, m, $\underline{CH}Me_2$), 2.45 (2H, m, \underline{CH}_2^iPr), 3.85 (3H, s, $CO\underline{CH}_3$), 4.59 (2H, s, \underline{CH}_2Ph), 5.15 (1H, m, \underline{CH}), 6.7-7.25 (5H, m, (MeO)Ar H).

δ_C (101 MHz, $CDCl_3$) 22.88 (\underline{CH}_3), 22.95 (\underline{CH}_3), 26.75 ($\underline{CH}Me_2$), 44.90 (\underline{CH}_2), 50.24 (\underline{CH}_2Ar), 57.32 ($O\underline{CH}_3$), 11456-13288 (Ar CH), 155.42 (Ar C), 160.54 ($=\underline{CH}$).

LRMS m/z (ESI) 206.2 $[M+H]^+$.

(E)-N-(2-methylpropylidene)-1-phenylmethanamine 349



Benzylamine (5.0 g, 47 mmol), and isovaleraldehyde (3.9 g, 48 mmol), were added to toluene (100ml) and the resulting solution was heated under reflux overnight, while

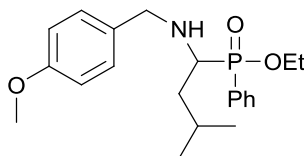
water was removed by a using Dean-Stark apparatus. Once the reaction was complete the solvent was removed *in vacuo* to give the title compound as a yellow oil. (7.0 g, 43 mmol, 92% yield) which was used immediately in the next step without further purification.

δ_H (400 MHz, $CDCl_3$) 1.06 (6H, d, J 7.0, \underline{CH}_3), 2.50 (1H, m, $\underline{CH}Me_2$), 4.55 (2H, s, \underline{CH}_2Ph), 7.21-7.37 (5H, m, Ar H), 7.43 (1H, d, J 5, $\underline{CH}CHMe_2$).

δ_C (101 MHz, $CDCl_3$) 21.7 (CH_3), 24.6 ($CHMe_2$), 36.5 ($CHMe_2$), 67.0 (CH_2Ph), 129.10 (Ar CH), 130.21 (Ar CH), 130.80 (Ar CH), 130.92 (Ar CH), 141.65 (Ar CH), 173.40 ($=CH$);

LRMS m/z (ESI) 162 $[M+H]^+$.

[1-(4-Methoxy-benzylamino)-3-methyl-butyl]-phenyl-phosphinic acid ethyl ester
350



Triethylamine (7.7 ml, 55 mmol) was added to a stirred solution of compound **320** (8.5 g, 50 mmol) in dichloromethane (150 ml) maintained at 0 °C under a dry nitrogen atmosphere. After 15 min $TMSCl$ (6.95 ml, 55 mmol) was slowly added (Caution! Excessive fuming) and the reaction mixture was left stirring for a further 15 min. Imine **348** (10.8 g, 53 mmol) was added and the reaction mixture was left to warm up to room temperature overnight under a dry nitrogen atmosphere. Once the reaction was complete the reaction mixture was poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na_2SO_4 and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 6:1 - 2:1) to give a

diastereomeric mixture of *the title compound* (18.8 g, 50 mmol, quant. yield), as a colourless oil.

$\nu_{\text{max}}/\text{cm}^{-1}$ 2868-2957 1611 1514 1465 1438 1249 1178 1120 1022

δ_{P} (162 MHz, CDCl_3) 44.05, 44.29

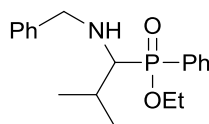
δ_{H} (400 MHz, CDCl_3) 0.63 (3H, d, J 6.5, CH_3), 0.75 (3H, d, J 6.5, CH_3), 0.80 (3H, d, J 6.7, CH_3), 0.87 (3H, d, J 6.6, CH_3), 1.31 (3H, t, J 5.4, OCH_2CH_3), 1.33 (3H, t, J 5.4, OCH_2CH_3), 1.36-1.44 (2H, m, CH_2^iPr), 1.56-1.62 (2H, m, CH_2^iPr), 1.71-1.84 (2H, m, 2x CHMe_2), 2.99-3.06 (2H, m, 2x NHCH), 3.69 (2H, d, J 1.6, CH_2Ar), 3.76 (3H, s, COCH_3), 3.78 (3H, s, COCH_3), 3.88 (2H, d, J 12.0, CH_2Ar), 3.86-3.93 (2H, m, OCH_2CH_3), 4.06-4.17 (2H, m, OCH_2CH_3), 6.76 (2H, d, J 8.5, (MeO)Ar H), 6.82 (2H, d, J 8.4, (MeO)Ar H), 7.04 (2H, d, J 7.4, (MeO)Ar H), 7.22 (2H, d, J 8.4, (MeO)Ar H), 7.45-7.49 (4H, m, Ar H), 7.53-7.57 (2H, m, Ar H), 7.78-7.85 (4H, m, Ar H).

δ_{C} (101 MHz, CDCl_3) 16.70 (d, J_{P} 6.3, OCH_2CH_3), 16.78 (d, J_{P} 6.1, OCH_3), 21.28 (CH_3), 21.61 (CH_3), 23.48 (CH_3), 23.50 (CH_3), 24.43 (d, J_{P} 11.6, CHMe_2), 24.62 (d, J_{P} 10.7, CHMe_2), 38.20 (CH_2), 38.51 (CH_2), 51.72 (CH_2NAr), 51.75 (CH_2NAr), 53.74 (d, J_{P} 37.2, NHCH), 54.46 (d, J_{P} 32.6, NHCH), 55.38 (OCH_3), 55.40 (OCH_3), 60.94 (d, J_{P} 7.3, OCH_2CH_3), 60.98 (d, J_{P} 7.3, OCH_2CH_3), 113.75 (2x MeOAr CH), 113.80 (2x MeOAr CH), 128.28 (d, J_{P} 13.3, Ar CH), 128.56 (d, J_{P} 11.9, Ar CH), 128.65 (d, J_{P} 11.8, Ar CH), 129.70 (2x MeOAr CH), 129.88 (2x MeOAr CH), 130.87 (d, J_{P} 111.5, Ar C), 130.89 (d, J_{P} 111.2, Ar C), 132.35 (d, J_{P} 2.8, Ar CH), 132.40 (d, J_{P} 2.8, Ar CH), 132.49 (d, J_{P} 9.3, Ar CH), 132.71 (d, J_{P} 9.3, Ar CH), 158.81 (MeOAr C), 158.86 (MeOAr C).

LRMS m/z (ESI) 376.3 $[\text{M}+\text{H}]^+$.

HRMS calcd. for $\text{C}_{21}\text{H}_{31}\text{O}_3\text{NP}$ 376.2036, found 376.2029 $[\text{MH}]^+$.

(1-Benzylamino-2-methyl-propyl)-phenyl-phosphinic acid ethyl ester 351



Triethylamine (18 ml, 130 mmol) was added to a stirred solution of compound **320** (20 g, 118 mmol) in dichloromethane (200 ml) maintained at 0 °C under a dry nitrogen atmosphere. After 15 min TMSCl (16.5 ml, 130 mmol) was slowly added (Caution! Excessive fuming) and the reaction mixture was left stirring for a further 15 min. Imine **349** (19 g, 118 mmol) was added and the stirred reaction mixture was left to warm up to room temperature overnight under a dry nitrogen atmosphere. Once the reaction was complete the reaction mixture was poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over MgSO₄ and stripped of solvent *in vacuo* to give the crude title compound (37.08 g, 112 mmol, 95% crude yield). Recrystallisation using EtOAc gave colourless crystals.

Diastereomer 1 (**351a**) (Crystal) (7 g, 18% yield).

mp 100-103 °C.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3281 3058 2861-2972 1603 1590 1496 1461 1451 1435 1389 1374 1364 1216
1165 1116 1093 1070 1024

δ_{p} (243 MHz, CDCl₃) 45.12

δ_{H} (600 MHz, CDCl₃) 0.91 (3H, d, *J* 6.8, CH₃), 0.94 (3H, d, *J* 6.8, CH₃), 1.32 (3H, t, *J* 7.0, OCH₂CH₃), 1.82-1.89 (2H, m, CHMe₂ + NH), 2.84 (1H, dd, *J* 3.1, 9.9, NHCH), 3.79-3.86 (1H, m OCH₂CH₃), 3.90 (2H, dd, *J* 12.8, 43.6, CH₂Ar), 4.09-4.16 (1H, m, OCH₂CH₃), 7.21-7.25 (1H, m, Ar H), 7.27-7.29 (4H, m, Ar H), 7.45-7.48 (2H, m, Ar H), 7.53-7.56 (1H, m, Ar H), 7.78-7.82 (2H, m, Ar H).

δ_C (151 MHz, $CDCl_3$) 16.83 (d, J_P 6.3, OCH_2CH_3), 17.35 (d, J_P 2.8, CH_3) 21.49 (d, J_P 14.1, CH_3), 28.46 (d, J_P 8.3, $CHMe_2$) 53.88 (d, J_P 3.9, CH_2Ar), 60.52 (d, J_P 7.5, OCH_2CH_3) 61.73 (d, J_P 100.2, PCH), 127.10 (Ar CH), 128.30 (Ar CH), 128.65 (d, J_P 11.9, Ar CH), 128.67 (Ar CH), 131.2 (d, J_P 111.0, Ar C), 132.22 (d, J_P 2.9, Ar CH), 132.33 (d, J_P 9.6, Ar CH), 140.46 (Ar C).

LRMS m/z (ESI) 332.17 $[M+H]^+$.

HRMS (ESI) calcd. for $C_{19}H_{27}O_2NP$ 332.1774, found 332.1779 $[M+H]^+$.

Diastereomer 2 (**351b**) (Oil) (10 g, 30% yield):

ν_{max}/cm^{-1} 3316 3282 3054 2872-2959 1603 1495 1454 1436 1389 1374 1322 1281 1228
1192 1127 1047 1024

δ_P (243 MHz, $CDCl_3$) 42.15

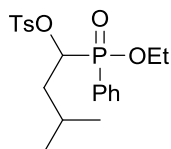
δ_H (600 MHz, $CDCl_3$) 0.93 (3H, d, J 6.9, $\underline{CH_3}$), 0.98 (3H, d, J 6.8, $\underline{CH_3}$), 1.21 (3H, t, J 7.1, $OCH_2\underline{CH_3}$), 2.18-2.28 (2H, m, $\underline{CHMe_2}$), 2.75-2.79 (1H, m, \underline{PCH}), 3.70-3.78 (1H, m, $OCH_2\underline{CH_3}$), 3.84 (2H, dd, J 10.3, 28.8, $\underline{CH_2Ar}$), 3.96-4.09 (1H, m, $OCH_2\underline{CH_3}$), 6.94-6.96 (1H, m, Ar H), 7.10-7.12 (2H, m, Ar H), 7.21-7.23 (2H, m, Ar H), 7.38-7.42 (2H, m, Ar H), 7.45-7.50 (1H, m, Ar H), 7.71-7.79 (2H, m, Ar H).

δ_C (151 MHz, $CDCl_3$) 16.55 (d, J_P 6.2, OCH_2CH_3), 18.15 (d, J_P 3.8, CH_3) 21.35 (d, J_P 11.4, CH_3), 28.36 (d, J_P 8.1, $CHMe_2$), 53.63 (d, J_P 5.7 CH_2Ar), 60.57 (d, J_P 7.6, CH_2), 60.89 (d, J_P 108.3, PCH), 126.92 (Ar CH), 128.10 (Ar CH), 128.34 (Ar CH), 128.46 (d, J_P 11.8, Ar CH), 131.09 (d, J_P 111.6, Ar C), 132.05 (d, J_P 2.7, Ar CH), 132.42 (d, J_P 9.5, Ar CH), 139.87 (Ar C).

LRMS m/z (ESI) 332.17 $[M+H]^+$.

HRMS (ESI) calcd. for $C_{19}H_{27}O_2NP$ 332.1774, found 332.1779 $[M+H]^+$.

Toluene-4-sulfonic acid 1-(ethoxy-phenyl-phosphinoyl)-3-methyl-butyl ester 352



TsCl (9.54 g, 50.05 mmol) was added to a stirred solution of compound **347** (3.66 g, 14.3 mmol) in pyridine (60 ml) maintained at 0 °C. Once addition was complete the reaction was left to stir for 4 h and monitored by TLC. Once the reaction was complete the solvent was removed *in vacuo* and the residue was diluted with dichloromethane and poured into water (50 ml) and the organic layer was extracted. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na₂SO₄ and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 4:1 - 1:1) to give *the title compound* (3.79 g, 9.25 mmol, 65% yield) as a colourless oil.

$\nu_{\max}/\text{cm}^{-1}$ 3473 2871-2958 1596 1439 1362 1230 1189 1174 1121 1095 1022

δ_{P} (243 MHz, CDCl₃) 35.10

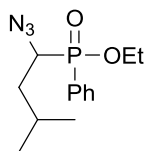
δ_{H} (600 MHz, CDCl₃) 0.85 (3H, d, *J* 6.2, CH₃), 0.87 (3H, d, *J* 6.2, CH₃), 1.28 (3H, t, *J* 7.1, OCH₂CH₃), 1.61-1.81 (3H, m, CH₂ⁱPr + CHMe₂), 2.41(3H, s, ArCH₃), 3.94-4.04 (1H, m, OCH₂CH₃), 4.04-4.13 (1H, m, OCH₂CH₃), 4.95-4.99 (1H, m, PCH), 7.22-7.24 (2H, m, MeAr H), 7.38-7.43 (2H, m, MeAr H), 7.53-7.57 (1H, m, Ar H), 7.62-7.68 (4H, m, Ar H).

δ_{C} (151 MHz, CDCl₃) 16.43 (d, *J*_P 5.9, OCH₂CH₃), 21.38 (CH₃), 21.62 (CH₃), 23.05 (ArCH₃), 24.11 (d, *J*_P 9.3, CHMe₂), 61.80 (d, *J*_P 6.6, OCH₂CH₃), 77.31 (d, *J*_P 117.7, PCH), 127.65 (d, *J*_P 128.4, Ar C), 127.80 (MeAr CH), 128.58 (d, *J*_P 12.8, Ar CH), 129.53 (MeAr CH), 132.67 (d, *J*_P 9.7, Ar CH), 132.91 (d, *J*_P 2.8, Ar CH), 133.89 (MeAr C), 144.63 (MeAr C).

LRMS m/z (ESI) 411.2 $[M+H]^+$.

HRMS (ESI) calcd. for $C_{20}H_{27}O_5PS$ 411.1390, found 411.1391 $[M+H]^+$.

(1-Azido-3-methyl-butyl)-phenyl-phosphinic acid ethyl ester 353



Triphenylphosphine (3.82 g, 15 mmol) followed by sodium azide (1.14 g, 17 mmol) were added to a stirred solution of compound **347** (3.73 g, 15 mmol) in CCl_4 (50 ml) followed by heating at 60 °C for 3 h. Once the reaction was complete the solvent was removed *in vacuo* and the residue was diluted with dichloromethane and poured into water (10 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 20 ml). The combined organic extracts were dried over Na_2SO_4 and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 6:1 - 3:1) to give *the title compound* (3.94 g, 14.03 mmol, 96% yield) as a colourless oil.

ν_{max}/cm^{-1} 3462 2871-2958 2101 1592 1468 1438 1389 1369 1289 1247 1224 1121 1022

δ_P (162 MHz, $CDCl_3$) 39.29.

δ_H (400 MHz, $CDCl_3$) 0.94 (6H, d, J 6.6, $\underline{CH_3}$), 1.31-1.37 (1H, m, $\underline{CH_2}^iPr$), 1.40 (3H, t, J 7.1, $OCH_2\underline{CH_3}$), 1.54-1.62 (1H, m, $\underline{CH_2}^iPr$), 1.77-1.87 (1H, m, $\underline{CHMe_2}$), 3.68-3.75 (1H, dt, J 2.9, 9.5, \underline{PCH}), 4.0-4.10 (1H, m, $O\underline{CH_2}CH_3$), 4.17-4.27 (1H, m, $O\underline{CH_2}CH_3$), 7.52-7.57 (2H, m, Ar H), 7.62-7.66 (2H, m, Ar H), 7.84-7.89 (2H, m, Ar H).

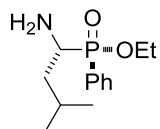
δ_C (101 MHz, $CDCl_3$) 16.57 (d, J_P 6.0, OCH_2CH_3), 20.83 (CH_3), 22.53 (CH_3), 23.18 (CH_3), 25.04 (d, J_P 13.0, $CHMe_2$), 36.09 ($\underline{CH_2}^iPr$), 58.11 (d, J_P 110, CH), 61.71 (d, J_P

6.7, OCH₂CH₃), 127.49 (d, *J*_P 124.0, Ar C), 128.76 (d, *J*_P 12.0, 2 x Ar CH), 132.66 (d, *J*_P 10.0, 2 x Ar CH), 133.06 (d, *J*_P 3.0, Ar CH).

LRMS *m/z* (ESI) 304.2 [M+Na]⁺, 282.2 [M+H]⁺.

HRMS (ESI) calcd. for C₁₃H₂₁O₂N₃P 282.1366, found 282.1362 [M+H]⁺.

1-Amino-3-methyl-butyl)-phenyl-phosphinic acid ethyl ester **354a**¹¹²



A mixture of compound **353** (3.94 g, 14 mmol) and 5% Pd/C (0.79 g, 20 % wt) in EtOAc was stirred under a hydrogen atmosphere overnight. The catalyst was filtered through celite and the solvent was removed *in vacuo* to give *the title compound* (3.58 g, 14 mmol, quant. yield) as a yellow oil.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3375 2868-2955 1672 1592 1549 1467 1438 1387 1367 1259 1201 1121 1023

δ_{P} (162 MHz, CDCl₃) 44.05

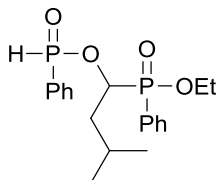
δ_{H} (400 MHz, CDCl₃) 0.80 (3H, d, *J* 6.6, CH₃), 0.87 (3H, d, *J* 6.7, CHMe₂), 1.26 (3H, t, *J* 7.1, OCH₂CH₃), 1.28-1.35 (2H, m, NH₂), 1.45-1.53 (1H, m, CH₂ⁱPr), 1.77-1.87 (1H, m, CHMe₂), 3.07 (1H, ddd, *J* 3.4, 8.0, *J*_P 11.3, PCH) 3.80-3.90 (1H, m, OCH₂CH₃), 4.02-4.11 (1H, m, OCH₂CH₃), 7.41-7.47 (2H, m, Ar H), 7.49-7.54 (1H, m, Ar H), 7.72-7.77 (2H, m, Ar H).

δ_{C} (101 MHz, CDCl₃) 16.56 (d, *J*_P 6.0, OCH₂CH₃), 20.93 (CH₃), 23.62 (CH₃), 24.13 (d, *J*_P 12.0, CHMe₂), 38.68 (CH₂ⁱPr), 48.98 (d, *J*_P 106.0, PCH), 61.08 (d, *J*_P 7.4, OCH₂CH₃), 128.77 (d, *J*_P 116.0, Ar C), 128.63 (d, *J*_P 12.0, 2 x Ar CH), 132.43 (d, *J*_P 2.0, Ar CH), 132.55 (d, *J*_P 9.0, Ar CH).

LRMS *m/z* (ESI) 278.3 [M+Na]⁺, 256.3 [M+H]⁺.

HRMS (ESI) calcd. for C₁₃H₂₃O₂N₁P 256.1461, found 256.1464 [M+H]⁺.

Ethyl (3-methyl-1-((phenylhydrophosphoryl)oxy)butyl)(phenyl)phosphinate 359-360



Triphosgene (3.47 g, 12 mmol), followed by pyridine (2.85 ml, 35 mmol) were added to a stirred solution of compound **347** (9 g, 35 mmol) in dichloromethane (150 ml) maintained at 0 °C and under a dry nitrogen atmosphere. The mixture was left stirring for 3 h at 0 °C. Pyridine (3.21 ml, 40 mmol) was slowly added to the stirred solution of the chloroformate and phenylphosphinic acid (5.63 g, 40 mmol). (Caution! Exothermic reaction; Rapid Effervescence). Once the effervescence had stopped the stirred solution was left to warm up to room temperature under a dry nitrogen atmosphere. The solution was poured into 0.1M hydrochloric acid (50 ml) and the organic layer was separated. The organic extracts were washed with water (50 ml) and dried using Na₂SO₄. The combined organic extracts were stripped of solvent *in vacuo* to give *the title compound* as a colourless oil (14 g, quant. yield).

$\nu_{\max}/\text{cm}^{-1}$ 3400 (OH), 2840-2951 (CH), 1448, 1405, 1113 (P=O), 1017 (P=O)

δ_{P} (162 MHz, CDCl₃) 28.25 (d, *J* 8.8), 36.9 (d, *J* 8.8).

δ_{H} (400 MHz, CDCl₃) 0.45 (3H, d, *J* 6.6, CH₃), 0.51 (3H, d, *J* 6.4, CH₃), 1.00 (3H, t, *J* 7.1, OCH₂CH₃), 1.25-1.34 (1H, m, CHMe₂), 1.35-1.51 (2H, m, CH₂ⁱPr), 3.67-3.77 (1H, m, OCH₂CH₃), 3.79-3.90 (1H, m, OCH₂CH₃), 4.51-4.60 (1H, m, PCH), 7.06-7.46 (10H, m, Ar H), 7.32 (1H, d, *J* 575.0, PH).

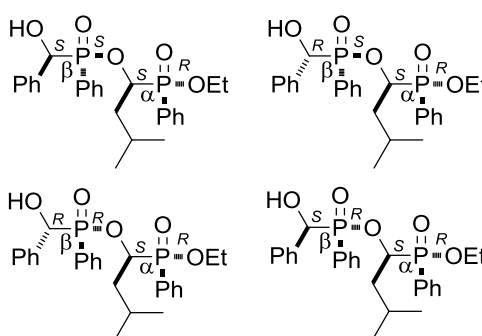
δ_{C} (101 MHz, CDCl₃) 16.78 (d, *J*_P 5.7, OCH₂CH₃), 21.19 (CH₃), 23.76 (CH₃), 24.17 (d, *J*_P 13.6, CHMe₂), 39.72 (d, *J*_P 3.6, CH₂), 62.04 (d, *J*_P 7.3, OCH₂CH₃), 68.01 (d, *J*_P

116.6, PCH), 127.81 (d, J_P 119.5, Ar C), 128.80 (d, J_P 1.8, Ar CH), 128.95 (d, J_P 3.6, Ar CH), 130.82 (d, J_P 12.0, Ar CH), 132.04 (d, J_P 133.4, Ar C), 132.61 (d, J_P 9.4, Ar CH), 132.91 (d, J_P 2.8, Ar CH), 133.03 (d, J_P 2.6, Ar CH).

LRMS m/z (ESI) 403.1 $[M+Na]^+$.

HRMS (ESI) calcd. for $C_{19}H_{26}O_4P_2Na$ 403.1204, found 403.1220 $[M+Na]^+$.

Ethyl (1-(((hydroxy(phenyl)methyl)(phenyl)phosphoryl)oxy)-3-methylbutyl)(phenyl)phosphinate 365



Triethylamine (6.7 ml, 48 mmol) was added to a stirred solution of compound **359-360** (10.2 g, 40 mmol) in dichloromethane (150 ml) maintained at 0 °C under a dry nitrogen atmosphere. After 15 min $TMSCl$ (6.1 ml, 48 mmol) was slowly added (Caution! Excessive fuming) and the reaction mixture was left stirring for a further 15 min. Benzaldehyde (4.9 ml, 48 mmol) was added and the reaction mixture was left to warm up to room temperature overnight under a dry nitrogen atmosphere. Once the reaction was complete the reaction mixture was poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na_2SO_4 and stripped of solvent *in vacuo* to give compound **362** (22 g, 40 mmol, quant. yield) as a yellow oil. TBAF (20 ml of a 1M solution in THF) was added to a stirred solution of this crude product in THF (20 ml). After 5 min, the reaction mixture was poured into water (60 ml) and was extracted with dichloromethane (3 x 60 ml). The combined organic extracts were dried over Na_2SO_4 and stripped of solvent *in vacuo* to give the title compound as a mixture of

diastereomers. The residue was subjected to chromatography (EtOAc : pet. ether 2:1, then 1:1, then neat EtOAc) the appropriate fractions were combined, stripped of solvent and crystallised, if appropriate, to afford:

Compound **365a** colourless crystals, (R_f (tlc) 0.62 in ether), 3.0 g (16% over three steps); mp 150-155 °C.

Found: C, 64.08; H, 6.57. $C_{26}H_{32}O_5P_2$ requires C, 64.19; H, 6.63.

$\nu_{\max}/\text{cm}^{-1}$ 3265 OH, 2854, 2923 CH, 1592, 1485, 1463, 1448, 1439, 1371, 1249, 1231, 1188, 1173, 1123 (P=O), 1056.

δ_P (162 MHz, CDCl_3) 40.65 (d, J 14.0), 43.15 (d, J 14.0).

δ_H (400 MHz, CDCl_3) 0.8 (3H, d, J 6.7, CH_3), 1.0 (3H, d, J 6.4, CH_3), 1.30-1.45 (2H, m, CH_2^iPr), 1.5 (3H, t, J 7.0, OCH_2CH_3), 1.77-1.90 (1H, m, CHMe_2), 4.19-4.38 (3H, m, OCH_2CH_3 and P^βCH), 5.18 (1H, ddd, J 2.9, 11.2, J_P 22.0, P^αCH), 5.50 (1H, d, J_P 14.0, OH), 7.15-7.17 (3H, m, Ar H), 7.22-7.28 (4H, m, Ar H), 7.40-7.47 (3H, m, Ar H), 7.59-7.64 (2H, m, Ar H), 7.67-7.71 (1H, m, Ar H), 7.90-7.95 (2H, m, Ar H).

δ_C (101 MHz, CDCl_3) 16.65 (d, J_P 5.7, OCH_2CH_3), 21.36 (CH_3), 23.48 (CH_3), 24.05 (d, J_P 11.9, CHMe_2), 41.22 (CH_2), 62.63 (d, J_P 6.8, OCH_2CH_3), 71.18 (dd, J_P 7.5, 121.6, PCH), 72.60 (d, J_P 101.4, PCH), 126.27 (d, J_P 127.2, Ar C), 126.69 (d, J_P 5.0, Ar CH), 126.96 (d, J_P 129.0, Ar C), 127.15 (d, J_P 3.5, Ar CH), 127.58 (d, J_P 12.3, Ar CH), 127.65 (d, J_P 2.7, Ar CH), 128.85 (d, J_P 12.7, Ar CH), 132.21 (d, J_P 2.7, Ar CH), 132.32 (d, J_P 8.8, Ar CH), 132.84 (d, J_P 9.6, Ar CH), 133.46 (d, J_P 2.8, Ar CH), 136.04 (d, J_P 2.2, Ar C).

LRMS m/z (ESI) 509.2 $[\text{M}+\text{Na}]^+$, 487.2 $[\text{M}+\text{H}]^+$.

HRMS (ESI) calcd. for $C_{26}H_{32}O_5P_2\text{Na}$ 509.1617, found 509.1609 $[\text{M}+\text{Na}]^+$.

Compound **365b** colourless crystals, (R_f (tlc) 0.59 in ether), 2.5 g (13% over three steps); mp 120-125 °C.

Found: C, 64.08; H, 6.55. $C_{26}H_{32}O_5P_2$ requires C, 64.19; H, 6.63.

$\nu_{\max}/\text{cm}^{-1}$ 3236 OH, 2853, 2924 CH, 1590, 1492, 1461, 1441, 1377, 1236, 1221, 1187 (P=O), 1055

δ_P (162 MHz, $CDCl_3$) 39.8 (d, J_P 14.6), 40.65 (d, J_P 14.8).

δ_H (400 MHz, $CDCl_3$) 0.74 (3H, d, J 6.7, $\underline{CH_3}$), 0.9 (3H, d, J 6.4, $\underline{CH_3}$), 1.29-1.43 (2H, m, $\underline{CH_2}^i\text{Pr}$), 1.47 (3H, t, J 7.0, OCH_2CH_3), 1.57-1.62 (1H, m, $\underline{CH}Me_2$), 4.19-4.34 (3H, m, OCH_2CH_3 and $P^\beta\underline{CH}$), 5.25 (1H, ddd, J 3.1, 10.7, J_P 21.8, $P^\alpha\underline{CH}$), 7.22 (1H, s, \underline{OH}), 7.21-7.23 (4H, m, Ar H), 7.30-7.33 (2H, m, Ar H), 7.32-7.37 (2H, m, Ar H), 7.44-7.49 (3H, m, Ar H), 7.59-7.64 (2H, m, Ar H), 7.68-7.72 (1H, m, Ar H), 7.89-7.94 (2H, m, Ar H).

δ_C (101 MHz, $CDCl_3$) 16.64 (d, J_P 5.5, OCH_2CH_3), 20.91 (CH_3), 23.40 (CH_3), 23.84 (d, J_P 12.1, CH), 40.95 (CH_2), 62.54 (d, J_P 6.7, OCH_2CH_3), 71.29 (dd, J_P 7.6, 120.0, PCH), 74.36 (d, J_P 100.3 PCH), 126.66 (d, J_P 128.1, Ar C), 127.72 (d, J_P 3.1, Ar CH), 127.88 (d, J_P 5.2, Ar CH), 127.89 (d, J_P 2.8, Ar CH), 128.01 (d, J_P 12.4, Ar CH), 128.79 (d, J_P 12.7, Ar CH), 129.33 (d, J_P 125.7, Ar C), 131.68 (d, J_P 9.1, Ar CH), 132.30 (d, J_P 2.6, Ar CH), 132.77 (d, J_P 9.5, Ar CH), 133.30 (d, J_P 2.8, Ar CH), 136.05 (Ar C).

LRMS m/z (ESI) 509.2 $[M+Na]^+$, 487.2 $[M+H]^+$.

HRMS (ESI) calcd. for $C_{26}H_{32}O_5P_2Na$ 509.1617, found 509.1622 $[M+Na]^+$.

Compound **365c** and **365d** were isolated together as a 1:1 mixture after chromatography and crystallisation. (R_f (tlc) 0.2 in ether), 1 g (7% over three steps);

$\nu_{\text{max}}/\text{cm}^{-1}$ 3263 3061 2869-2958 1714 1592 1493 1469 1439 1389 1369 1216 1120 1056
1025

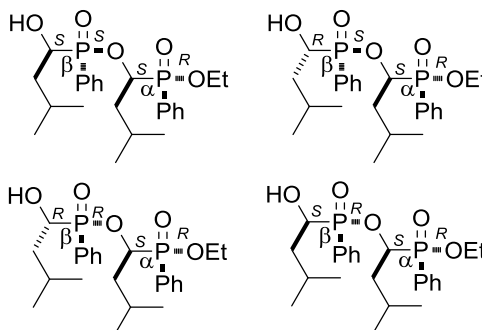
δ_{P} (243 MHz, CDCl_3) 37.84 (d, J_{P} 13.6), 40.82 (d, J_{P} 13.6), and 38.51 (d, J_{P} 14.7),
40.38 (d, J_{P} 14.7).

δ_{H} (600 MHz, CDCl_3) 0.50 (3H, d, J 6.5, CH_3), 0.61 (6H, d, J 6.5, 2x CH_3), 0.70 (3H, d, J 6.6, CH_3), 0.80 (3H, t, J 7.0, OCH_2CH_3), 0.86 (3H, t, J 7.1, OCH_2CH_3), 1.10-1.16 (1H, m, CH_2^{iPr}), 1.28-1.36 (1H, m, CH_2^{iPr}), 1.40-1.45 (1H, m, CH_2^{iPr}), 1.56-1.62 (1H, m, CHMe_2), 1.64-1.69 (1H, m, CHMe_2), 1.70-1.75 (1H, m, CH_2^{iPr}), 3.51-3.56 (1H, m, OCH_2CH_3), 3.57-3.62 (2H, m, OCH_2CH_3), 3.63-3.70 (1H, m, OCH_2CH_3), 4.64-4.69 (1H, m, $\text{P}^{\beta}\text{CH}$), 4.70-4.74 (1H, m, $\text{P}^{\beta}\text{CH}$), 5.08 (1H, d, J 9.5, $\text{P}^{\alpha}\text{CH}$), 5.12 (1H, d, J 9.6, $\text{P}^{\alpha}\text{CH}$), 7.13-7.18 (6H, m, Ar H), 7.21-7.22 (2H, m, Ar H), 7.25-7.29 (4H, m, Ar H), 7.31-7.36 (4H, m, Ar H), 7.37-7.39 (2H, m, Ar H), 7.40-7.43 (2H, m, Ar H), 7.44-7.50 (2H, m, Ar H), 7.58-7.64 (6H, m, Ar H), 7.72-7.78 (2H, m, Ar H).

δ_{C} (151 MHz, CDCl_3) 15.82 (d, J_{P} 6.3, OCH_2CH_3), 15.92 (d, J_{P} 6.2, OCH_2CH_3), 20.95 (CH_3), 21.08 (CH_3), 23.09 (CH_3), 23.12 (CH_3), 23.53 (d, J_{P} 11.1, CH), 23.92 (d, J_{P} 10.7, CH), 39.39 (CH_2), 39.42 (CH_2), 61.31 (d, J_{P} 6.8, OCH_2CH_3), 61.46 (d, J_{P} 6.8, OCH_2CH_3), 71.95 (dd, J_{P} 9.8, 119.8, PCH), 72.01 (dd, J_{P} 10.1, 119.4, PCH), 73.80 (d, J_{P} 111.0, PCH), 73.88 (d, J_{P} 112.0, PCH), 127.17 (d, J_{P} 5.0, Ar CH), 127.51 (d, J_{P} 124.4, Ar C), 127.52 (d, J_{P} 121.6, Ar C), 127.62 (d, J_{P} 4.9, Ar CH), 127.67 (d, J_{P} 124.0, Ar C), 127.68 (d, J_{P} 120.0, Ar C), 127.75 (d, J_{P} 3.3, Ar CH), 127.85 (d, J_{P} 2.5, Ar CH), 127.90 (d, J_{P} 8.4, Ar CH), 128.10 (d, J_{P} 5.5, Ar CH), 128.26 (d, J_{P} 6.9, Ar CH), 128.48 (d, J_{P} 12.1, Ar CH), 128.69 (d, J_{P} 12.4, Ar CH), 128.79 (d, J_{P} 12.5, Ar CH), 132.35 (d, J_{P} 2.7, Ar CH), 132.46 (d, J_{P} 9.9, Ar CH), 132.48 (d, J_{P} 9.6, Ar CH), 132.50 (d, J_{P} 9.5, Ar CH), 132.93 (d, J_{P} 3.8, Ar CH), 133.01 (d, J_{P} 3.1, Ar CH), 133.00 (d, J_{P} 9.6, Ar CH), 133.15 (d, J_{P} 9.5, Ar CH), 136.04 (Ar C), 136.37 (Ar C).

LRMS m/z (ESI) 509.2 $[M+Na]^+$, 487.2 $[M+H]^+$.

Ethyl (1-(((1-hydroxy-3-methylbutyl)(phenyl)phosphoryl)oxy)-3-methylbutyl)(phenyl)phosphinate 366



Triethylamine (10 ml, 72 mmol) was added to a stirred solution of compound **359-360** (14 g, 40 mmol) in dichloromethane (150 ml) maintained at 0 °C under a dry nitrogen atmosphere. After 15 min TMSCl (9.1 ml, 72 mmol) was slowly added (Caution! Excessive fuming) and the reaction mixture was left stirring for a further 15 min. Isovaleraldehyde (4.3 ml, 40 mmol) was added and the reaction mixture was left to warm up to room temperature overnight under a dry nitrogen atmosphere. Once the reaction was complete the reaction mixture was poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na₂SO₄ and stripped of solvent *in vacuo* to give compound **363** (21 g, 40 mmol, quant. yield) as a yellow oil. TBAF (20 mL of a 1M solution in THF) was added to a stirred solution of this crude product in THF (20 ml). After 5 min, the reaction mixture was poured into water (60 ml) and was extracted with dichloromethane (3 x 60 ml). The combined organic extracts were dried over Na₂SO₄ and stripped of solvent *in vacuo* to give the title compound as a mixture of diastereomers. The residue was subjected to chromatography (EtOAc : Pet. Ether 2:1, then 1:1, then neat EtOAc) the appropriate fractions were combined, stripped of solvent and crystallised, if appropriate, to afford:

Compound **366a** colourless oil, (R_f (tlc) 0.7 in ether), 3 g (18% over three steps);

Found: C, 61.89; H, 7.67. C₂₄H₃₆O₅P₂ requires C, 61.79; H, 7.78.

$\nu_{\max}/\text{cm}^{-1}$ 3294 OH, 2869, 2957 (CH), 1592, 1468, 1439, 1387, 1368, 1300, 1249, 1218, 1122 (P=O)

δ_{P} (162 MHz, CDCl₃) 40.25 (d, J_{P} 14), 46.05 (d, J_{P} 14)

δ_{H} (400 MHz, CDCl₃) 0.65 (3H, d, J 7.0, CH₃), 0.70 (3H, d, J 7.0, CH₃), 0.75 (3H, d, J 7.0, CH₃), 0.80 (3H, d, J 7.0, CH₃), 1.17-1.27 (2H, m, CH₂ⁱPr), 1.34 (3H, t, J 7.0, OCH₂CH₃), 1.70-1.79 (1H, m, CHMe₂), 1.84-1.94 (1H, m, CHMe₂), 4.05-4.20 (2H, q, J_{H} 7.0, OCH₂CH₃), 4.29-4.38 (1H, ddd, J 2.6, 10.4, J_{P} 22.2, P ^{β} CH), 4.97 (1H, ddd, J 2.8, 10.4, J_{P} 22.2, P ^{α} CH), 6.16 (1H, dt, J_{P} 1.5, 4.2, OH), 7.42-7.52 (4H, m, Ar H), 7.57-7.63 (2H, m, Ar H), 7.72-7.77 (2H, m, Ar H), 7.98-8.03 (2H, m, Ar H).

δ_{C} (101 MHz, CDCl₃) 17.3 (d, J_{P} 6.0, OCH₂CH₃), 21.82 (CH₃), 21.88 (CH₃), 23.84 (CH₃), 24.36 (CH₃), 25.5 (CH), 25.8 (CH), 40.8 (CH₂), 41.71 (CH₂), 64.3 (CH₂), 73 (d, J_{P} 112.0, CH), 73 (d, J_{P} 117.0, PCH), 126.1 (d, J_{P} 127.0, Ar C), 127.9 (d, J_{P} 126.0, Ar C), 128.1 (d, J_{P} 12.2, 2 x Ar H), 128.8 (d, J_{P} 12.6, 2 x Ar CH), 132.2 (d, J_{P} 8.3, 2 x Ar CH), 132.3 (d, J_{P} 2.6, Ar CH), 132.8 (d, J_{P} 9.6, 2 x Ar CH), 133.3 (d, J_{P} 2.7, Ar CH).

LRMS m/z (ESI) 467.3 [M+H]⁺.

HRMS (ESI) calcd. for C₂₄H₃₆O₅P₂Na 489.1936, found 489.1923 [M+Na]⁺.

Compound **366b** colourless crystals, (R_{f} (tlc) 0.4 in ether), 3 g (18% over three steps); mp 90-95 °C.

Found: C, 61.84; H, 7.68. C₂₄H₃₆O₅P₂ requires C, 61.79; H, 7.78.

$\nu_{\max}/\text{cm}^{-1}$ 3284 OH, 2720-3053 CH, 1589, 1466, 1441, 1414, 1388, 1377, 1366, 1340, 1306, 1230, 1194, 1161, 1121 (P=O), 1077, 1034.

δ_{P} (162 MHz, CDCl₃) 41.0 (d, J_{P} 14), 42.1 (d, J_{P} 14).

δ_{H} (400 MHz, CDCl_3) 0.65 (3H, d, J 7.0, CH_3), 0.74 (3H, d, J 7.0, CH_3), 0.83 (3H, d, J 7.0, CH_3), 0.86 (3H, d, J 7.0, CH_3), 1.16-1.34 and 1.76-1.86 (4H, m, 2 x CH_2^{iPr}), 1.34 (3H, t, J 7.0, OCH_2CH_3), 1.52-1.64 (1H, m, CHMe_2), 1.86-1.94 (1H, m, CHMe_2), 4.00-4.19 (3H, m, OCH_2CH_3 and PCHb), 5.15 (1H, ddd, J_{HHP} 2.9, 11.3, 21.6, PCHa), 5.92 (1H, dd, J_{PH} 5.8, 1, OH), 7.31-7.80 (10H, m, Ar H).

δ_{C} (101 MHz, CDCl_3) 16.6 (d, J_{P} 6.0 OCH_2CH_3), 20.9 (CH_3), 21.1 (CH_3), 23.4 (CH_3), 23.6 (CH_3), 23.7 (d, J_{P} 11.0, CH), 24.2 (d, J_{P} 12.0, CH), 38.8 (d, J_{P} 3.0, CH_2), 41.1 (d, J_{P} 2.0, CH_2), 62.4 (d, J_{P} 7.0, OCH_2CH_3), 69.3 (d, J_{P} 105.0, PCH), 71.3 (dd, J_{P} 8.0, 112.0, PCH), 127.0 (d, J_{P} 128.0, Ar C), 128.9 (d, J_{P} 128.0, Ar C), 128.4 (d, J_{P} 12.2, 2 x Ar CH), 128.7 (d, J_{P} 12.6, 2 x Ar CH), 131.2 (d, J_{P} 9.2, 2 x Ar CH), 132.3 (d, J_{P} 2.3, Ar CH), 132.6 (d, J_{P} 11.6, 2 x Ar CH), 133.2 (d, J_{P} 2.5, Ar CH).

LRMS m/z (ESI) 489.2 $[\text{M}+\text{Na}]^+$, 467.2 $[\text{M}+\text{H}]^+$.

HRMS (ESI) calcd. for $\text{C}_{24}\text{H}_{36}\text{O}_5\text{P}_2\text{Na}$ 489.1936, found 489.1921 $[\text{M}+\text{Na}]^+$.

Compound **366c** white amorphous solid (R_{f} (tlc) 0.25 in ether) 2.5 g (15% over three steps).

mp 120-125 °C.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3280 OH, 2853, 2924 (CH), 1591, 1461, 1443, 1377, 1234, 1182, 1121 P=O, 1073.

δ_{P} (162 MHz, CDCl_3) 38.8 (d, J_{P} 14), 41.8 (d, J_{P} 14).

δ_{H} (400 MHz, CDCl_3) 0.51 (3H, d, J 7.0, CH_3), 0.74 (3H, d, J 7.0, CH_3), 0.92 (6H, d, J 7.0, CH_3), 0.94 (3H, d, J 7.0, CH_3), 1.18 (3H, t, J 7.0, OCH_2CH_3), 1.20-1.95 (6H, m, 2x CHMe_2 + 2x CH_2^{iPr}), 3.95 (2H, quint, J 7.0, J_{P} 7.0, OCH_2CH_3), 4.24 (1H, m, $\text{P}^{\beta}\text{CH}$), 4.35 (1H, bs, OH), 4.55 (1H, ddd, J_{P} 3.6, 9.7, J_{P} 17.8, $\text{P}^{\alpha}\text{CH}$), 7.48-7.52 (4H, m, Ar H), 7.57-7.63 (2H, m, Ar H), 7.77-7.78 (2H, m, Ar H), 7.99-8.06 (2H, m, Ar H).

δ_C (101 MHz, $CDCl_3$) 16.3 (d, J_P 6.0, CH_3), 20.9 (CH_3), 21.2 (CH_3), 23.1 (CH_3), 23.5 (CH_3), 23.7 (d, J_P 11.0, CH), 24.3 (d, J_P 14.0, CH), 38.90 (d, J_P 3.0, CH_2), 40.17 (CH_2), 61.8 (d, J_P 7.0, OCH_2CH_3), 70.0 (d, J_P 115.0, PCH), 72.7 (dd, J_P 11.0, 121.0, PCH) 126.7 (d, J_P 112.0, Ar C), 127.1 (d, J_P 126.0, Ar C), 128.4 (d, J_P 12.2, 2 x Ar CH), 128.7 (d, J_P 12.6, 2 x Ar CH), 132.6 (d, J_P 9.4, 2 x Ar CH), 132.7 (d, J_P 2.3, Ar CH), 133.1 (d, J_P 9.2, 2 x Ar CH), 133.1 (d, J_P 2.2, Ar CH).

LRMS m/z (ESI) 489.2 $[M+Na]^+$, 467.2 $[M+H]^+$.

HRMS (ESI) calcd. for $C_{24}H_{36}O_5P_2Na$ 489.1936, found 489.1920 $[M+Na]^+$.

Compound **366d** colourless crystals (R_f (tlc) 0.24 in ether) 0.2 g (1%);

mp 101-107 °C.

ν_{max}/cm^{-1} 3300 OH, 2868, 2957 CH, 1591, 1468, 1439, 1387, 1304, 1221, 1119 (P=O), 1070, 1025

δ_P (162 MHz, $CDCl_3$) 38.2 (d, J_P 14), 41 (d, J_P 14).

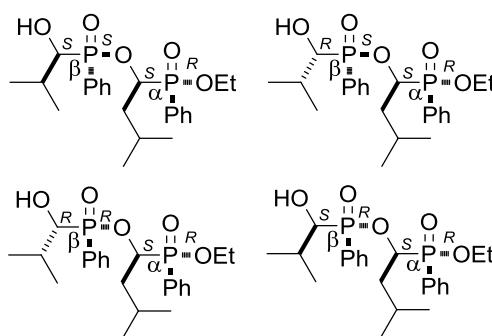
δ_H (400 MHz, $CDCl_3$) 0.55 (3H, d, J 7.0, $\underline{CH_3}$), 0.75 (3H, d, J 7.0, $\underline{CH_3}$), 0.92 (6H, d, J 7.0, $\underline{CH_3}$), 0.92 (3H, d, J 7.0, $\underline{CH_3}$), 1.12 (3H, t, J 7.0, $OCH_2\underline{CH_3}$), 1.30-2.0 (6H, m, 2 x $\underline{CHMe_2}$ and $\underline{CH_2}^iPr$), 3.9 (2H, quint, J_{HP} 7.0, 7.0, $OCH_2\underline{CH_3}$), 4.1 (1H, m, $P^\beta\underline{CH}$), 4.2 (1H, bs, \underline{OH}), 4.62 (1H, ddd, J_P 3.6, 9.8, J_P 17, $P^\alpha\underline{CH}$), 7.46-7.49 (4H, m, Ar H), 7.55-7.59 (2H, m, Ar H), 7.72-7.75 (2H, m, Ar H), 7.99-8.04 (2H, m, Ar H).

δ_C (101 MHz, $CDCl_3$) 16.2 (d, J_P 5.8, CH_3), 20.9 (CH_3), 21 (CH_3), 23.2 (CH_3), 23.7 (CH_3), 23.8 (d, J_P 11.0, CH), 24.3 (d, J_P 13.0, CH), 39.25 (CH_2), 40.12 (CH_2), 61.75 (d, J_P 6.5, OCH_2CH_3), 69.55 (d, J_P 112.0, PCH), 72.6 (dd, J_P 11.0, 122.0, PCH) 127.2 (d, J_P 98.0, Ar C), 127.9 (d, J_P 86.0, Ar C), 128.5 (d, J_P 12.3, 2 x Ar CH), 128.8 (d, J_P 13.0, 2 x Ar CH), 132.7 (d, J_P 2.3, Ar CH), 132.78 (d, J_P 9.4, 2 x Ar CH), 133.1 (d, J_P 9.4, 2 x Ar CH), 133.21 (d, J_P 2.2, Ar CH).

LRMS m/z (ESI) 489.2 $[M+Na]^+$, 467.2 $[M+H]^+$.

HRMS (ESI) calcd. for $C_{24}H_{36}O_5P_2Na$ 489.1936, found 489.1927 $[M+Na]^+$.

Ethyl (1-(((1-hydroxy-2-methylpropyl)(phenyl)phosphoryl)oxy)-3-methylbutyl)(phenyl)phosphinate 367



Triethylamine (6.7 ml, 48 mmol) was added to a stirred solution of compound **359-360** (10.2 g, 40 mmol) in dichloromethane (150 ml) maintained at 0 °C under a dry nitrogen atmosphere. After 15 min TMSCl (6.1 ml, 48 mmol) was slowly added (Caution! Excessive fuming) and the reaction mixture was left stirring for a further 15 min. Isobutyraldehyde (4.4 ml, 48 mmol) was added and the reaction mixture was left to warm up to room temperature overnight under a dry nitrogen atmosphere. Once the reaction was complete the reaction mixture was poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na_2SO_4 and stripped of solvent *in vacuo* to give compound **364** (21 g, 40 mmol, quant. yield) as a colourless oil. TBAF (20 ml of a 1M solution in THF) was added to a stirred solution of this crude product in THF (20 ml). After 5 min, the reaction mixture was poured into water (60 ml) and was extracted with dichloromethane (3 x 60 ml). The combined organic extracts were dried over Na_2SO_4 and stripped of solvent *in vacuo* to give the title compound (g, mmol) as a . The residue was subjected to chromatography (EtOAc : Pet. Ether 2:1,

then 1:1, then neat EtOAc) the appropriate fractions were combined, stripped of solvent and crystallised, if appropriate, to afford:

Compound **367a** colourless crystals, (R_f (tlc) 0.6 in ether), 0.5 g (3% over three steps); mp 104-107 °C.

$\nu_{\max}/\text{cm}^{-1}$ 3303 (OH), 2868, 2955 CH, 1593, 1488, 1438, 1390, 1339, 1225, 1198, 1126 (P=O), 1047

δ_P (162 MHz, CDCl_3) 41.26 (d, J_P 14.6), 45.23 (d, J_P 14.6).

δ_H (400 MHz, CDCl_3) 0.73 (3H, d, J 6.7, CH_3), 0.86 (3H, d, J 6.5, CH_3), 0.95 (3H, d, J 6.5, CH_3), 1.04 (3H, d, J 6.7, CH_3), 1.21-1.31 (2H, m, CH_2^iPr), 1.39 (3H, t, J 7.0, OCH_2CH_3), 1.62-1.70 (1H, m, CHCHMe_2), 1.71-1.79 (1H, m, CH_2CHMe_2), 3.95 (1H, ddd, J 3.2, 4.9, J_P 13, P^βCH), 4.09-4.17 (1H, m, OCH_2CH_3), 4.18-4.25 (1H, m, OCH_2CH_3), 4.96 (1H, ddd, J_{HHP} 2.6, 11.3, 23.6, P^αCH), 6.2 (1H, br s, OH), 7.35-7.39 (2H, m, Ar H), 7.45-7.48 (1H, m, Ar H), 7.49-7.53 (2H, m, Ar H), 7.57-7.61 (1H, m, Ar H), 7.78-7.87 (4H, m, Ar H).

δ_C (101 MHz, CDCl_3) 16.7 (d, J_P 5.8, OCH_2CH_3), 19.4 (d, J_P 11.0, CH_3), 20 (d, J_P 5.0, CH_3), 21.5 (CH_3), 23.5 (CH_3), 23.7 (d, J_P 12.0, CHMe_2), 29.8 (d, J_P 7.0, CHMe_2), 41.3 (CH_2), 62.5 (d, J_P 6.5, OCH_2CH_3), 70.9 (dd, J_P 7.2, 121.0, PCH), 74.7 (d, J_P 103.0, PCH), 126.3 (d, J_P 126.0, Ar C), 128.2 (d, J_P 11.6, 2 x Ar CH), 128.8 (d, J_P 13.0, 2 x Ar CH), 129.2 (d, J_P 126.0, Ar C), 132.0 (d, J_P 9.4, 2 x Ar CH), 132.4 (d, J_P 2.2, Ar CH), 132.85 (d, J_P 10.1, 2 x Ar CH), 133.4 (d, J_P 2.2, Ar CH).

LRMS m/z (ESI) 475.4 $[\text{M}+\text{Na}]^+$, 453.4 $[\text{M}+\text{H}]^+$.

HRMS (ESI) calcd. for $\text{C}_{23}\text{H}_{35}\text{O}_5\text{P}_2$ 453.1954, found 453.1962 $[\text{M}+\text{H}]^+$.

Compound **367b** colourless crystals, (R_f (tlc) 0.55 in ether), 0.4 g (2% over three steps); mp 93-97 °C.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3235 OH, 2870, 2955 CH, 1591, 1440, 1390, 1321, 1223, 1194, 1123 (P=O), 1051.

δ_{P} (162 MHz, CDCl_3) 41.57 (d, J_{P} 15.5), 42.55 (d, J_{P} 15.3).

δ_{H} (400 MHz, CDCl_3) 0.67 (3H, d, J 6.7, CH_3), 0.90 (3H, d, J 6.4, CH_3), 0.94 (3H, d, J 6.7, CH_3), 1.09 (3H, d, J 6.7, CH_3), 1.16-1.25 (1H, m, CH_2^{iPr}), 1.28-1.35 (1H, m, CH_2^{iPr}), 1.38 (3H, t, J 7.0, OCH_2CH_3), 1.48-1.57 (1H, m, CHMe_2), 1.72-1.81 (1H, m, CH_2CHMe_2), 3.92 (1H, ddd, J 1.0, 4.0, J_{P} 14.0, $\text{P}^{\beta}\text{CH}$), 4.12-4.19 (1H, m, OCH_2CH_3), 4.19-4.25 (1H, m, OCH_2CH_3), 5.23 (1H, ddd, J 2.2, 10.7, J_{P} 21.8, $\text{P}^{\alpha}\text{CH}$), 6.1 (1H, br s, OH), 7.37-7.42 (2H, m, Ar H), 7.47-7.51 (1H, m, Ar H), 7.52-7.56 (2H, m, Ar H), 7.6-7.64 (1H, m, Ar H), 7.66-7.71 (2H, m, Ar H), 7.8-7.85 (2H, m, Ar H).

δ_{C} (101 MHz, CDCl_3) 16.7 (d, J_{P} 5.1, OCH_2CH_3), 17.1 (d, J_{P} 4.3, CH_3), 20.8 (d, J_{P} 13.0, CH_3), 21.1 (CH_3), 23.5 (CH_3), 23.9 (d, J_{P} 12.3, CHMe_2), 29.4 (d, J_{P} 5.8, CHMe_2), 41.1 (CH_2), 62.5 (d, J_{P} 6.5, OCH_2CH_3), 70.9 (dd, J_{P} 8.0, 121.0, PCH), 75.8 (d, J_{P} 103.0, PCH), 126.9 (d, J_{P} 128.0, Ar C), 128.5 (d, J_{P} 12.3, 2 x Ar CH), 128.8 (d, J_{P} 13.0, 2 x Ar CH), 131 (d, J_{P} 118.0, Ar C), 131.2 (d, J_{P} 9.4, 2 x Ar CH), 132.3 (d, J_{P} 2.2, Ar CH), 132.8 (d, J_{P} 9.4, 2 x Ar CH), 133.3 (d, J_{P} 2.2, Ar CH).

LRMS m/z (ESI) 475.2 $[\text{M}+\text{Na}]^+$, 453.2 $[\text{M}+\text{H}]^+$.

HRMS (ESI) calcd. for $\text{C}_{23}\text{H}_{35}\text{O}_5\text{P}_2$ 453.1954, found 453.1960 $[\text{M}+\text{H}]^+$.

Compound 367c and 367d were isolated together after chromatography and crystallisation. ($R_{\text{f}}(\text{tlc})$ 0.35 in ether), 1.5 g (9% over three steps);

mp 122-126 °C.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3306 (OH), 2923-2854 (CH), 1590, 1463, 1377, 1236 (P=O), 1222 (P=O)

δ_{P} (243 MHz, CDCl_3) 39.06 (1P, d, J_{P} 17.8), 39.53 (1P, d, J_{P} 19.3), 41.81 (1P, d, J_{P} 19.3), 41.81 (1P, J_{P} 17.8).

δ_{H} (600 MHz, CDCl_3) 0.46 (3H, d, J 6.5, CH_3), 0.53 (3H, d, J 6.5, CH_3), 0.70 (6H, d, J 6.6, CH_3), 0.88 (3H, d, J 6.7, CH_3), 1.01 (6H, d, J 6.8, CH_3), 1.02 (3H, d, J 6.8, CH_3), 1.08 (3H, t, J 7.1, OCH_2CH_3), 1.15 (3H, t, J 7.1, OCH_2CH_3), 1.24-1.31 (2H, m, 2x CH_2^{iPr}), 1.49-1.55 (1H, m, CH_2^{iPr}), 1.55-1.62 (1H, m, CH_2^{iPr}), 1.68-1.75 (1H, m, CH_2CHMe_2), 1.76-1.83 (1H, m, CH_2CHMe_2), 1.97 (1H, hex, J_{P} 6.9, CHCHMe_2), 2.03 (1H, hex, J 6.9, CHCHMe_2), 3.79 (1H, dd, J 1.9, J_{P} 5.3, PCH), 3.82-3.87 (2H, m, OCH_2CH_3), 3.88-3.95 (3H, m, OCH_2CH_3 + PCHCH_2), 4.04 (1H, br s, OH), 4.44-4.49 (1H, m, PCHCH), 4.50-4.55 (1H, m, PCHCH), 4.57 (1H, br s, OH), 7.42-7.47 (8H, m, Ar H), 7.52-7.58 (4H, m, Ar H), 7.68-7.72 (4H, m, Ar H), 7.94-8.00 (4H, m, Ar H).

δ_{C} (151 MHz, CDCl_3) 16.33 (d, J_{P} 5.8, OCH_2CH_3), 16.43 (d, J_{P} 5.7, OCH_2CH_3), 17.60 (d, J_{P} 5.5, CH_3), 18.45 (d, J_{P} 8.5, CH_3), 20.25 (d, J_{P} 10.8, CH_3), 20.57 (d, J_{P} 10.8, CH_3), 21.15 (d, J_{P} 25.8, CH_3) 23.15 (d, J_{P} 17.6, CH_3) 23.80 (d, J_{P} 10.1, CHMe_2), 23.87 (d, J_{P} 10.8, CMe_2), 29.69 (d, J_{P} 1.6, CMe_2), 29.72 (d, J_{P} 3.1, CMe_2), 40.39 (CH_2), 40.40 (CH_2), 61.74 (d, J_{P} 6.7, OCH_2CH_3), 61.90 (d, J_{P} 6.7, OCH_2CH_3), 72.78 (dd, J_{P} 10.9, 120.9, PCH), 72.85 (dd, J_{P} 11.3, 121.2, PCH), 76.03 (d, J_{P} 94.5, PCH), 76.76 (d, J_{P} 98.8, PCH), 126.85 (d, J_{P} 86.8, Ar C), 127.35 (d, J_{P} 102.4, Ar C), 127.81 (d, J_{P} 111.0, Ar C), 128.01 (d, J_{P} 96.1, Ar C), 128.53 (d, J_{P} 8.3, Ar CH), 128.61 (d, J_{P} 8.6, Ar CH), 128.80 (d, J_{P} 3.1, Ar CH), 128.88 (d, J_{P} 3.4, Ar CH), 132.74 (d, J_{P} 8.7, 2x Ar CH), 132.75 (d, J_{P} 9.5, 2x Ar CH), 132.80 (d, J_{P} 5.8, Ar CH), 133.20 (d, J_{P} 2.8, Ar CH), 133.24 (d, J_{P} 2.8, Ar CH), 133.32 (d, J_{P} 9.1, Ar CH).

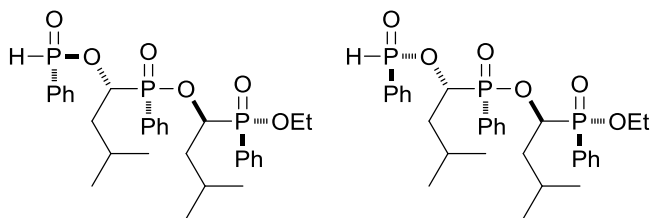
LRMS m/z (ESI) 453 $[\text{M}+\text{H}]^+$.

HRMS (ESI) calcd. for $\text{C}_{22}\text{H}_{35}\text{O}_5\text{P}_2$ 453.1954, found 453.1939 $[\text{M}+\text{H}]^+$.

Ethyl

(3-methyl-1-(((3-methyl-1-

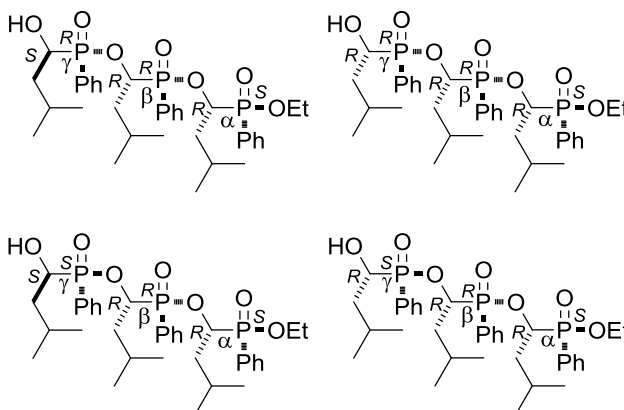
((phenylhydrophosphoryl)oxy)butyl)(phenyl)phosphoryl)oxy)butyl)(phenyl)phosphinite 369



Triphosgene (0.45 g, 2 mmol), followed by pyridine (0.36 ml, 5 mmol) were added to a stirred solution of compound **366b** (1.93 g, 4 mmol) in dichloromethane (100ml) maintained at 0 °C and under a dry nitrogen atmosphere. The mixture was left stirring for 3 h at 0 °C. Pyridine (0.36 ml, 4.5 mmol) was slowly added to the stirred solution of the chloroformate and phenylphosphinic acid (0.64g, 5 mmol). (Caution! Exothermic reaction; Rapid Effervescence). Once the effervescence had stopped the stirred solution was refluxed for 45 min. until the reaction was complete. The solution was poured into 0.1M hydrochloric acid (50 ml) and the organic layer was separated. The organic extracts were washed with water (50 ml) and dried using Na₂SO₄. The combined organic extracts were stripped of solvent in vacuo to give the crude *title compound* as a colourless oil (2.45 g, 4.14 mmol, quant. yield).

Ethyl

(1-(((1-(((1-hydroxy-3-methylbutyl)(phenyl)phosphoryl)oxy)-3-methylbutyl)(phenyl)phosphoryl)oxy)-3-methylbutyl)(phenyl)phosphinate 371



Triethylamine (0.64 ml, 4.6 mmol) was added to a stirred solution of compound **369** (2.45 g, 4.1 mmol) in dichloromethane (150 ml) maintained at 0 °C under a dry nitrogen atmosphere. After 15 min TMSCl (0.58 ml, 4.6 mmol) was slowly added (Caution! Excessive fuming) and the reaction mixture was left stirring for a further 15 min. Isovaleraldehyde (0.56 ml, 4.6 mmol) was added and the reaction mixture was left to warm up to room temperature overnight under a dry nitrogen atmosphere. Once the reaction was complete the reaction mixture was poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na₂SO₄ and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 5:1 - 2:1) to give compound **370b** (0.1 g, 0.13 mmol, 3% yield) as a colourless oil, containing a single diastereomer. Compound **371b** was deprotected using SiO₂ to give *the title compound* (0.09 g, 0.13 mmol, quant. yield) as a colourless oil.

Compound **371b**.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3296 OH, 2899, 2958 CH, 1592, 1469, 1437, 1389, 1303, 1228, 1121 (P=O), 1050.

δ_{P} (162 MHz, CDCl₃) 36.91 (d, J_{P} 15.8, P $^{\gamma}$), 41.56 (dd, J_{P} 15.8, J_{P} 22.1, P $^{\beta}$), 42.52 (d, J_{P} 22.1, P $^{\alpha}$).

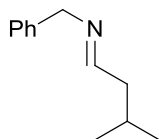
δ_H (400 MHz, $CDCl_3$) 0.51 (3H, d, J 6.0, $\underline{CH_3}$), 0.64 (3H, d, J 6.5, $\underline{CH_3}$), 0.67 (3H, d, 6.5, $\underline{CH_3}$), 0.75 (3H, d, 6.5, $\underline{CH_3}$), 0.87 (3H, d, 6.5, $\underline{CH_3}$), 0.91 (3H, d, J 6.0, $\underline{CH_3}$), 1.31 (3H, t, J 7.0, $OCH_2\underline{CH_3}$), 1.33-1.62 (7H, m, 3 x $\underline{CH_2}$ and $\underline{CHMe_2}$), 1.71-1.78 (1H, m, $\underline{CHMe_2}$), 1.92-1.99 (1H, m, $\underline{CHMe_2}$), 4.09-4.20 (2H, m, $OCH_2\underline{CH_3}$), 4.32 (1H, apparent t, J_P 11.0, J_P 10.0, $P^{\beta}\underline{CH}$), 4.74 (1H, ddd, J 3.6, 9.5, J_P 18, $P^{\alpha}\underline{CH}$), 5.02 (1H, ddd, J 3.1, 9.9, J_P 18, $P^{\gamma}\underline{CH}$), 5.64 (1H, bs, \underline{OH}), 7.37-7.62 (11H, m, Ar H), 7.65-7.7 (2H, m, Ar H), 7.75-7.8 (2H, m, Ar H), 8.04 8.10 (2H, m, Ar H).

δ_C (101 MHz, $CDCl_3$) 16.7 (d, J_P 5.0, OCH_2CH_3), 21.0 ($\underline{CH_3}$), 21.14 ($\underline{CH_3}$), 21.28 ($\underline{CH_3}$), 23.10 ($\underline{CH_3}$), 23.42 ($\underline{CH_3}$), 23.64 ($\underline{CH_3}$), 24.1 (d, J_P 12.0, CH), 24.3 (d, J_P 14.0, CH), 24.4 (d, J_P 14.0, CH), 39.3 ($\underline{CH_2}$), 40.2 ($\underline{CH_2}$), 40.5 ($\underline{CH_2}$), 62 (d, J_P 8.0, OCH_2CH_3), 69.8 (d, J_P 113.0, $P^{\gamma}\underline{CH}$), 72.43 (dd, J_P 9.0, J_P 121.0, PCH), 74.3 (dd, J_P 12.0, J_P 118.0, PCH), 126.3 (d, J_P 110.0, Ar C), 127.7 (d, J_P 126.0, Ar C), 128.1 (d, J_P 110.0, Ar C), 128.45 (d, J_P 8.0, Ar CH), 128.53 (d, J_P 9.0, Ar CH), 128.84 (d, J_P 13.0, Ar CH), 132.2-133.5 (overlapping 6 x d, 6 x Ar CH).

LRMS m/z (ESI) 699.4 $[M+Na]^+$, 677.5 $[M+H]^+$.

HRMS (ESI) calcd. for $C_{35}H_{52}O_7P_3$ 677.2920, found 677.2908 $[M+Na]^+$.

(E)-N-(3-methylbutylidene)-1-phenylmethanamine 379



Benzylamine (5.0 g, 47 mmol), and isovaleraldehyde (3.9 g, 48 mmol), were added to toluene (100ml) and the resulting solution was heated under reflux overnight, while water was removed by a using Dean-Stark apparatus. Once the reaction was complete the solvent was removed *in vacuo* to give the title compound as a yellow oil (7.9 g, 45

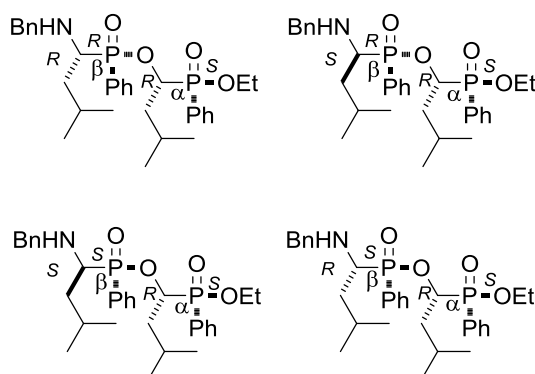
mmol, 96% yield) which was used immediately in the next step without further purification.

δ_H (400 MHz, $CDCl_3$) 0.98 (3H, d, J 7.0, $\underline{CH_3}$), 0.99 (3H, d, J 7.0, $\underline{CH_3}$), 1.96 (1H, m, $\underline{CHMe_2}$), 2.23 (2H, q, J 6.0, $\underline{CH_2}$), 4.59 (2H, s, $\underline{CH_2Ph}$), 5.15 (1H, t, J 5.2, \underline{CH}), 7.20-7.40 (5H, m, Ar H).

δ_C (101 MHz, $CDCl_3$) 22.64 ($\underline{CH_3}$), 22.68 ($\underline{CH_3}$), 26.48 ($\underline{CHMe_2}$), 44.90 ($\underline{CH_2}$), 65.46 ($\underline{CH_2Ph}$), 126.9-128.5 (Ar CH), 135.3 (Ar C), 165.85 ($=\underline{CH}$).

LRMS m/z (ESI) 176.0 $[M+H]^+$.

Ethyl **(1-(((1-(benzylamino)-3-methylbutyl)(phenyl)phosphoryl)oxy)-3-methylbutyl)(phenyl)phosphinate 380**



Triethylamine (5.2 ml, 37 mmol) was added to a stirred solution of compound **359-360** (8 g, 31 mmol) in dichloromethane (150 ml) maintained at 0 °C under a dry nitrogen atmosphere. After 15 min $TMSCl$ (4.7 ml, 37 mmol) was slowly added (Caution! Excessive fuming) and the reaction mixture was left stirring for a further 15 min. Imine **379** (4 ml, 37 mmol) was added and the reaction mixture was left to warm up to room temperature overnight under a dry nitrogen atmosphere. Once the reaction was complete the reaction mixture was poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na_2SO_4 and stripped of solvent *in vacuo*.

The residue was subjected to chromatography (EtOAc : Pet. Ether 4:1 - 1:0) to give *the title compound*.

Diastereomer 1 (**380a**) (Solid) (3.1 g, 18% yield):

mp 105-107 °C.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3330 3059 2868-2951 1593 1494 1468 1454 1438 1386 1368 1307 1237 1210
1190 1162 1119 1056 1022

δ_{P} (162 MHz, CDCl_3) 36.73 (d, J 14.87, P^{α}), 44.94 (d, J 14.86, P^{β}).

δ_{H} (400 MHz, CDCl_3) 0.64 (3H, d, J 6.5, CH_3), 0.83 (6H, d, J 6.6, CH_3), 0.85 (3H, d, J 6.5, CH_3), 0.94 (3H, t, J 7.1, OCH_2CH_3), 1.17-1.27 (1H, m, $\text{CH}_2^{\text{iPr}}\beta$), 1.34-1.48 (2H, m, 2x CH_2^{iPr}), 1.73-1.81 (1H, m, $\text{C}^{\alpha}\text{HMe}_2$), 1.92-2.03 (2H, m, $\text{C}^{\alpha}\text{HMe}_2 + \text{C}^{\alpha}\text{H}_2^{\text{iPr}}$), 3.02 (1H, td, J 3.1, J_{P} 10.9, $\text{P}^{\beta}\text{CH}$), 3.65-3.81 (2H, m, OCH_2CH_3), 4.04 (2H, q, J 13.0, CH_2Ph), 4.77-4.84 (1H, m, $\text{P}^{\alpha}\text{CH}$), 7.23-7.30 (2H, m, Ar H), 7.31-7.35 (3H, m, Ar H), 7.42-7.48 (4H, m, Ar H), 7.53-7.58 (2H, m, Ar H), 7.72-7.82 (4H, m, Ar H).

δ_{C} (101 MHz, CDCl_3) 15.92 (d, J_{P} 6.3, OCH_2CH_3), 20.94 (CH_3), 21.23 (CH_3), 23.05 (CH_3), 23.55 (CH_3), 24.14 (d, J_{P} 12.1, CHMe_2), 24.19 (d, J_{P} 10.7, CHMe_2), 38.92 (d, J_{P} 4.3, CH_2), 39.39 (t, J_{P} 2.6, CH_2), 52.39 (d, J_{P} 1.9, CH_2Ar), 55.24 (d, J_{P} 105.0, PCH), 61.08 (d, J_{P} 6.9, OCH_2CH_3), 71.16 (dd, J_{P} 9.6, 120.6, PCH), 126.97 (Ar CH), 128.14 (d, J_{P} 12.1, Ar CH), 128.23 (Ar CH), 128.54 (Ar CH), 128.64 (d, J_{P} 12.4, Ar CH), 128.58 (d, J_{P} 122.9, Ar C), 129.20 (d, J_{P} 125.1, Ar C), 132.07 (d, J_{P} 2.6, Ar CH), 132.58 (d, J_{P} 6.9, Ar CH), 132.67 (d, J_{P} 6.7, Ar CH), 132.77 (d, J_{P} 2.7, Ar CH), 140.32 (Ar C).

LRMS m/z (ESI) 556 $[\text{M}+\text{H}]^+$.

Diastereomer 2 (**380b**) (Oil) (2.5 g, 15% yield):

$\nu_{\text{max}}/\text{cm}^{-1}$ 3325 2850-2957 1590 1485 1460 1450 1430 1380 1307 1210 1180 1152 1120

δ_P (162 MHz, $CDCl_3$) 37.49 (d, J 15.96, P^a), 46.31 (d, J 16.19, P^b).

δ_H (400 MHz, $CDCl_3$) 0.50 (3H, d, J 7.1, $\underline{CH_3}$), 0.51 (3H, d, J 6.6, $\underline{CH_3}$), 0.62 (3H, d, J 6.5, $\underline{CH_3}$), 0.81 (3H, d, J 5.3, $\underline{CH_3}$), 1.27 (3H, t, J 7.1, $OCH_2\underline{CH_3}$), 1.29-1.41 (3H, m, $\underline{CHMe_2}$ + $\underline{CH_2}^iPr$), 1.44-1.55 (2H, m, $\underline{CH_2}^iPr$), 1.69-1.74 (1H, m, $\underline{CHMe_2}$), 2.97-3.03 (1H, m, P^bCH), 3.88 (2H, q, J 15.5, $\underline{CH_2}Ph$), 4.02-4.15 (2H, m, $OCH_2\underline{CH_3}$), 4.98-5.08 (1H, m, P^aCH), 7.13-7.16 (2H, m, Ar H), 7.19-7.26 (3H, m, Ar H), 7.32-7.45 (4H, m, Ar H), 7.47-7.52 (2H, m, Ar H), 7.70-7.79 (4H, m, Ar H).

δ_C (101 MHz, $CDCl_3$) 16.59 (d, J_P 5.9, OCH_2CH_3), 21.02 (CH_3), 21.24 (CH_3), 22.91 (CH_3), 23.52 (CH_3), 24.30 (d, J_P 11.2, $CHMe_2$), 24.59 (d, J_P 11.8, $CHMe_2$), 38.34 (d, J_P 4.2, CH_2), 38.53 (d, J_P 3.9, CH_2), 52.49 (d, J_P 3.4, CH_2Ar), 55.57 (d, J_P 102.8, PCH), 61.68 (d, J_P 6.4, OCH_2CH_3), 70.70 (dd, J_P 8.6, 121.7, PCH), 126.86 (Ar CH), 128.13 (d, J_P 12.1, Ar CH), 128.22 (Ar CH), 128.38 (Ar CH), 128.66 (d, J_P 12.4, Ar CH), 128.48 (d, J_P 124.0, Ar C), 130.79 (d, J_P 116.9, Ar C), 132.03 (d, J_P 5.8, Ar CH), 132.11 (d, J_P 6.6, Ar CH), 132.59 (d, J_P 2.1, Ar CH), 132.68 (d, J_P 2.2, Ar CH), 140.57 (Ar C).

LRMS m/z (ESI) 556 $[M+H]^+$.

Diastereomers 3 and 4 (380c-d) (Oil Mix) (1.5 g, 9% yield):

ν_{max}/cm^{-1} 3320 3055 2854-2965 1469 1430 1370 1237 1210 1180 1155 1116 1050

δ_P (162 MHz, $CDCl_3$) (1:0.86) 36.74 (d, J 17.7, P^a), 45.51 (d J 17.7, P^b) and 36.77 (d, J 14.8 P^a), 45.01 (d, J 14.8 P^b).

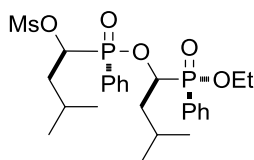
δ_H (400 MHz, $CDCl_3$) 0.77-0.92 (24H, m, $\underline{CH_3}$), 0.92 (3H, t, J 7.3, $OCH_2\underline{CH_3}$), 0.99 (3H, t, J 7.1, $OCH_2\underline{CH_3}$), 1.38-1.62 (5H, m, $\underline{CHMe_2}$ + $\underline{CH_2}^iPr$), 1.82-2.05 (5H, m, $\underline{CHMe_2}$ + $\underline{CH_2}^iPr$), 2.98-3.06 (2H, m, 2x P^bCH), 3.61-3.79 (4H, m, $\underline{CH_2}Ar$ + $OCH_2\underline{CH_3}$), 3.97-4.07 (4H, m, $\underline{CH_2}Ar$ + $OCH_2\underline{CH_3}$), 4.61-4.68 (1H, m, P^aCH), 4.76-

4.82 (1H, m, P^aCH), 7.08-7.11 (2H, m, Ar H), 7.17-7.25 (5H, m, Ar H), 7.26-7.34 (5H, m, Ar H), 7.40-7.59 (8H, m, Ar H), 7.71-7.85 (10H, m, Ar H).

δ_C (101 MHz, $CDCl_3$) 15.91 (d, J_P 6.3, OCH_2CH_3), 16.02 (d, J_P 6.4, OCH_2CH_3), 21.00 (CH_3), 21.22 (CH_3), 21.43 (CH_3), 21.44 (CH_3), 23.01 (CH_3), 23.04 (CH_3), 23.50 (CH_3), 23.52 (CH_3), 24.13 (d, J_P 12.0, $CHMe_2$), 24.17 (d, J_P 10.1, $CHMe_2$), 24.27 (d, J_P 10.2, $CHMe_2$), 24.38 (d, J_P 11.1, $CHMe_2$), 37.91 (d, J_P 2.4, CH_2), 38.90 (d, J_P 4.3, CH_2), 39.34 (d, J_P 2.3, CH_2), 39.73 (d, J_P 2.3, CH_2), 51.99 (d, J_P 4.4, CH_2Ar), 52.36 (d, J_P 2.0, CH_2Ar), 54.88 (d, J_P 108.1, PCH), 55.20 (d, J_P 105.0, PCH), 61.09 (d, J_P 6.9, OCH_2CH_3), 61.12 (d, J_P 6.8, OCH_2CH_3), 71.16 (dd, J_P 9.6, 120.5, PCH), 71.61 (dd, J_P 10.2, 121.1, PCH), 126.96 (Ar CH), 126.97 (Ar CH), 128.16 (d, J_P 14.1, Ar CH), 128.24 (d, J_P 9.0, Ar CH), 128.64 (d, J_P 12.6, Ar CH), 128.49 (d, J_P 8.5, Ar CH), 128.28 (Ar CH), 128.53 (Ar CH), 128.44 (d, J_P 119.3, Ar C), 128.51 (d, J_P 124.0, Ar C), 128.57 (d, J_P 123.6, Ar C), 129.73 (d, J_P 109.4, Ar C), 132.51 (d, J_P 3.8, Ar CH), 135.55 (d, J_P 5.4, Ar CH), 132.61 (d, J_P 5.5, Ar CH), 132.65 (d, J_P 3.0, Ar CH), 132.67 (d, J_P 2.0, Ar CH), 132.71 (d, J_P 4.7, Ar CH), 132.75 (d, J_P 4.5, Ar CH), 132.79 (d, J_P 2.7, Ar CH), 139.97 (Ar C), 140.26 (Ar C).

LRMS m/z (ESI) 556 $[M+H]^+$.

1-((1-(ethoxy(phenyl)phosphoryl)-3-methylbutoxy)(phenyl)phosphoryl)-3-methylbutyl methanesulfonate 383



Triethylamine (4 ml, 30 mmol) was added dropwise to a stirred solution of $MsCl$ (1.6 g, 20 mmol) and compound **366a** (0.94 g, 2 mmol) in dichloromethane (75 ml) maintained at 0 °C. Once the addition was complete the reaction was left to stir for 4 h and

monitored by TLC. Once the reaction was complete the mixture was poured into water (30 ml) and the organic layer was extracted. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na₂SO₄ and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 8:1 - 3:1) to give the title compound (1 g, 2 mmol, 91% yield) as a colourless oil.

$\nu_{\text{max}}/\text{cm}^{-1}$ 2871-2958 1591 1469 1439 1354 1228 1173 1119 1056

δ_{P} (162 MHz, CDCl₃) 36.60 (d, *J* 14.4, P^{*a*}), 37.67 (d, *J* 14.2, P^{*b*})

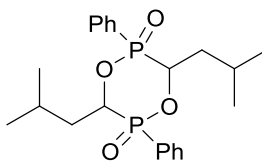
δ_{H} (400 MHz, CDCl₃) 0.44 (3H, d, *J* 6.4, CH₃), 0.48 (3H, d, *J* 6.5, CH₃), 0.85 (3H, d, *J* 6.7, CH₃), 0.89 (3H, d, *J* 6.5, CH₃), 1.03-1.10 (1H, m, CHMe₂), 1.12-1.17 (1H, m, CH₂^{*i*}Pr), 1.32 (3H, t, *J* 7.1, OCH₂CH₃), 1.50-1.56 (2H, m, CH₂^{*i*}Pr), 1.60-1.69 (1H, m, CH₂^{*i*}Pr), 1.79 (1H, sep, *J* 6.6, CHMe₂), 3.18 (3H, s, OSO₂CH₃), 3.93-4.01 (1H, m, OCH₂CH₃), 4.14-4.24 (1H, m, OCH₂CH₃), 4.95-5.02 (1H, m, P^{*a*}CH), 5.08-5.14 (1H, m, P^{*b*}CH), 7.42-7.50 (4H, m, Ar H), 7.53-7.58 (2H, m, Ar H), 7.73-7.82 (4H, m, Ar H).

δ_{C} (101 MHz, CDCl₃) 16.46 (d, *J*_P 6.5, OCH₂CH₃), 20.53 (CH₃), 20.91 (CH₃), 22.86 (CH₃), 23.37 (CH₃), 23.89 (d, *J*_P 11.7, CH), 24.26 (d, *J*_P 12.0, CH), 38.64 (d, *J*_P 3.9, CH₂), 38.72 (d, *J*_P 3.3, CH₂), 39.36 (CH₃), 62.11 (d, *J*_P 6.6, OCH₂CH₃), 70.99 (dd, *J*_P 7.9, 119.8, PCH), 72.19 (dd, *J*_P 8.0, 120.0, PCH), 78.27 (d, *J*_P 115.2, PCH), 127.60 (d, *J*_P 123.4, 2x Ar C), 128.62 (d, *J*_P 12.8, 2x Ar CH), 129.00 (d, *J*_P 12.5, 2x Ar CH), 132.35 (d, *J*_P 9.6, 2x Ar CH), 132.60 (d, *J*_P 9.7, 2x Ar CH), 133.29 (d, *J*_P 2.2, Ar CH), 133.32 (d, *J*_P 2.4, Ar CH).

LRMS *m/z* (ESI) 545.2 [M+H]⁺.

HRMS (ESI) calcd. for C₂₅H₃₉O₇P₂S 545.1886, found 545.1887 [M+H]⁺.

3,6-diisobutyl-2,5-diphenyl-1,4,2,5-dioxadiphosphinane 2,5-dioxide 385



Triphenylphosphine (0.89 g, 3 mmol) was added to a stirred solution of compound **366a** (0.75 g, 2 mmol) in CCl₄ (50 ml) followed by heating at 60 °C for 3 h. Once the reaction was complete the solvent was removed *in vacuo* and the residue was diluted with dichloromethane and poured into water (20 ml) and the organic layer was extracted. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na₂SO₄ and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 4:1 - 2:1) to give *the title compound* (0.68 g, 2 mmol, quant. yield) as a colourless solid, that after recrystallisation was submitted for X-ray crystallography.

mp 173-175 °C.

$\nu_{\max}/\text{cm}^{-1}$ 3064 2872-2959 1593 1486 1466 1438 1389 1372 1344 1299 1284 1233 1210
1155 1121 1065 1003

δ_{P} (162 MHz, CDCl₃) 29.28 (d, J 5.8, P ^{α}), 36.48 (d, J 5.8, P ^{β}).

δ_{H} (400 MHz, CDCl₃) 0.69 (3H, d, J 6.6, CH₃), 0.75 (3H, d, J 6.7, CH₃), 0.80 (3H, d, J 5.3, CH₃), 0.82 (3H, d, J 5.4, CH₃), 1.18 (1H, m, P ^{β} CH₂ⁱPr), 1.41 (1H, m, P ^{α} CH₂ⁱPr), 1.61 (1H, m, P ^{β} CH₂ⁱPr), 1.80 (2H, m, 2x CHMe₂), 2.17 (1H, m, P ^{α} CH₂ⁱPr), 4.80 (1H, m, P ^{β} CH), 5.11 (1H, m, P ^{α} CH), 7.47-7.52 (4H, m, Ar H), 7.57-7.62 (2H, m, Ar H), 7.7.79-7.84(2H, m, Ar H), 7.92-7.97(2H, m, Ar H).

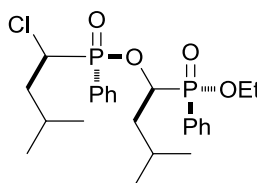
δ_{C} (101 MHz, CDCl₃) 20.97 (CH₃), 22.95 (CH₃), 23.93 (CH₃), 24.03 (CH₃), 37.7 (d, J_{P} 6.0, CHMe₂), 39.9 (CH₂ⁱPr), 70.36 (d, J_{P} 7.0, CH), 71.33 (d, J_{P} 7.0, CH), 74.69 (d, J_{P} 8.0, CH), 75.66 (d, J_{P} 8.0, CH), 125.54 (d, J_{P} 138.0, Ar C), 127.60 (d, J_{P} 131.0, Ar C),

128.8 (d, J_P 14.0, 2 x Ar CH), 129.2 (d, J_P 13.0, 2 x Ar CH), 131.5 (d, J_P 10.0, 2 x Ar CH), 132.9 (d, J_P 10.0, 2 x Ar CH), 133.7 (d, J_P 3.0, Ar CH), 133.8 (d, J_P 3.0, Ar CH).

LRMS m/z (ESI) 421.3 $[M+H]^+$, 420.2 $[M]^+$.

HRMS (ESI) calcd. for $C_{22}H_{30}O_4P_2$ 420.1614, found 420.1616 $[M]^+$.

Ethyl (1-(((1-chloro-3-methylbutyl)(phenyl)phosphoryl)oxy)-3-methylbutyl)(phenyl)phosphinate 387



Triphenylphosphine (0.56 g, 2 mmol) was added to a stirred solution of compound **366b** (0.2 g, 0.43 mmol) in CCl_4 (50 ml) followed by heating at 60 °C for 3 h. Once the reaction was complete the solvent was removed *in vacuo* and the residue was diluted with dichloromethane and poured into water (20 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na_2SO_4 and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 4:1 - 2:1) to give *the title compound* (0.18 g, 0.43 mmol, quant. yield) as a colourless oil.

ν_{max}/cm^{-1} 3484 2871-2958 1592 1469 1439 1388 1370 1233 1201 1120 1033

δ_P (162 MHz, $CDCl_3$) 36.92 (d, J 13.4, P^a), 40.55 (d, 13.4, P^b).

δ_H (400 MHz, $CDCl_3$) 0.58 (3H, d, J 6.6, $\underline{CH_3}$), 0.65 (3H, d J 6.4, $\underline{CH_3}$),

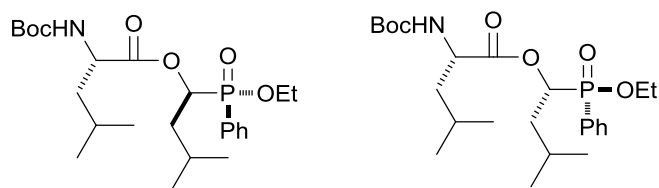
0.84 (6H, d, J 6.1, 2x $\underline{CH_3}$), 1.24-1.29 (1H, m, $\underline{CHMe_2}$), 1.37-1.42 (2H, m, $\underline{CH_2}^iPr$), 1.49-1.59 (2H, m, $\underline{CH_2}^iPr$), 1.75-1.85 (1H, m, $\underline{CHMe_2}$), 4.0-4.1 (1H, m, \underline{PCH}), 5.02-5.04 (1H, m, \underline{PCH}), 7.36-7.41 (2H, m, Ar H), 7.45-7.49 (3H, m, Ar H), 7.52-7.57 (1H, m, Ar H), 7.73-7.78 (2H, m, Ar H), 7.84-7.88 (2H, m, Ar H).

δ_C (101 MHz, $CDCl_3$) 16.65 (d, J_P 5.9, OCH_2CH_3), 20.39 (CH_3), 20.78 (CH_3), 23.17 (CH_3), 23.21 (CH_3), 24.20 (d, J_P 11.6, $CHMe_2$), 24.59 (d, J_P 12.3, $CHMe_2$), 39.5 (CH_2^iPr), 53.81 (d, J_P 103.4, PCH), 61.76 (d, J_P 6.6, OCH_2CH_3), 71.85 (dd, J_P 8.0, 121.2, PCH), 127.86 (d, J_P 106.6, Ar C), 127.58 (d, J_P 108.0, Ar C), 128.05 (d, J_P 12.9, Ar CH), 128.63 (d, J_P 12.5, Ar CH), 132.76 (d, J_P 3.8, Ar CH), 132.82 (d, J_P 9.3, Ar CH), 132.93 (d, J_P 9.7, Ar CH), 132.94 (d, J_P 2.8, Ar CH).

LRMS m/z (ESI) 488.3 $[M+H]^+$, 487.3 $[M+H]^+$, 485.3 $[M+H]^+$.

HRMS (ESI) calcd. for $C_{23}H_{36}O_4P_2^{35}Cl$ 484.1699, found 485.1754 $[M+H]^+$.

***(S)*-2-tert-Butoxycarbonylamino-4-methyl-pentanoate 1-(ethoxy-phenyl-phosphinoyl)-3-methyl-butyl ester 388**



DCC (3.3 g, 16 mmol), followed by DMAP (0.2 g, 2 mmol) were added to a stirred solution of compound **347** (2.05 g, 8 mmol) and Boc-(L)-Leu-OH (1.85 g, 8 mmol) in dichloromethane (100 ml) maintained at 0 °C. The mixture was left stirring for 3 h and the reaction was monitored by TLC. Once complete, the reaction mixture was filtered to remove DHU and then stripped of solvent *in vacuo*. The residue was diluted with dichloromethane and poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na_2SO_4 , stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 4:1 - 1:1) to give *the title compound* (3.03 g, 7 mmol, 81% yield) as a colourless oil.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3310, 2851-2929, 1738, 1706, 1625, 1575, 1530, 1469, 1449, 1438, 1389, 1365, 1310, 1284, 1243, 1222, 1201, 1161, 1120, 1089, 1019.

δ_{P} (162 MHz, CDCl_3) 36.84* 37.14 (* denotes the distinguishable signals due to the major isomer)

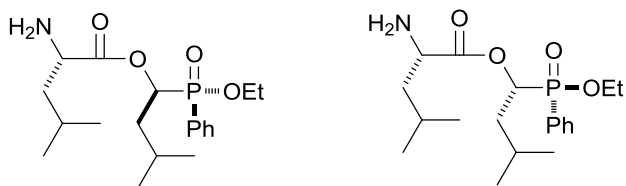
δ_{H} (400 MHz, CDCl_3) 0.80 (3H, d, J 6.0, CH_3), 0.81 (3H, d, J 8.70, CH_3), 0.86 (3H, d, J 2.7, CH_3), 0.87 (3H, d, J 3.5, CH_3), 0.90 (6H, d, J 5.7, 2 x CH_3), 0.92 (6H, d, J 7.2, 2 x CH_3), 1.31-1.34 (6H, m, 2x OCH_2CH_3), 1.41 [18H, 2x s, $\text{C}(\text{CH}_3)_3$], 1.46-1.59 (6H, m, 2x NHCHCH_2 + 2x $\text{NHCHCH}_2\text{CH}(\text{CH}_3)_2$), 1.64-1.68 (2H, m, 2x $\text{PCHCH}_2\text{CHMe}_2$), 1.76-1.84 (2H, m, PCHCH_2), 1.89-1.92 (2H, m, PCHCH_2), 3.96-4.04 (2H, m, OCH_2CH_3), 4.10-4.18 (2H, m, OCH_2CH_3), 4.23-4.27 (1H, m, NHCH^*), 4.28-4.32 (1H, m, NHCH), 4.74 (1H, d, J 9.0, NH^*), 4.79 (1H, d, J 8.8, NH), 5.41 (1H, d, J 11.4, PCH^*), 5.45-5.48 (1H, m, PCH), 7.46-7.49 (4H, m, Ar H), 7.53-7.57 (2H, m, Ar H), 7.75-7.79 (4H, m, Ar H).

δ_{C} (101 MHz, CDCl_3) 16.64 (d, J_{P} 7.0, OCH_2CH_3), 16.69 (d, J_{P} 6.1, OCH_2CH_3), 21.04 (CH_3), 21.14 (CH_3), 21.65 (CH_3), 21.78 (CH_3), 23.05 (CH_3), 23.19 (CH_3), 23.40 (CH_3), 23.42 (CH_3), 24.36 (CHMe_2), 24.44 (CHMe_2), 24.75 (CHMe_2), 24.84 (CHMe_2), 28.38 [$\text{C}(\text{CH}_3)_3$], 28.41 [$\text{C}(\text{CH}_3)_3$], 37.02 (CH_2), 37.42 (CH_2), 41.68 (CH_2), 41.86 (CH_2), 52.14 (NHCH), 52.33 (NHCH), 61.73 (d, J_{P} 7.0, 2x OCH_2CH_3), 69.56 (d, J_{P} 117.4, 2x PCH), 79.78 (OCMe_3), 79.93 (OCMe_3), 128.29 (d, J_{P} 125.7, Ar C), 128.42 (d, J_{P} 118.7, Ar C), 128.79 (d, J_{P} 12.9, Ar CH), 128.88 (d, J_{P} 12.8, Ar CH), 132.45 (d, J_{P} 9.8, Ar CH), 132.51 (d, J_{P} 9.9, Ar CH), 132.95 (d, J_{P} 2.9, Ar CH), 133.0 (d, J_{P} 2.5, Ar CH), 155.20 (NCO_2), 156.95 (NCO_2), 172.35 (CCO_2), 172.71 (CCO_2).

LRMS m/z (ESI) 487.4 $[\text{M}+\text{NH}_4]^+$, 470.3 $[\text{M}+\text{H}]^+$.

HRMS (ESI) calcd. for $\text{C}_{24}\text{H}_{41}\text{O}_6\text{NP}$ 470.2666, found 470.2663 $[\text{M}+\text{H}]^+$.

***(S)*-2-Amino-4-methylpentanoic acid 1-(ethoxyphenylphosphinoyl)-3-methylbutyl ester 389**



TFA (5 ml, 65 mmol) was added to a stirred solution of compound **388** (1.61 g, 3 mmol) in dichloromethane (50 ml) maintained at 0 °C for 1 h. On completion of the reaction the reaction mixture was poured into saturated NaHCO₃ (50 ml) and the organic layer was separated. The organic phase was washed with NaHCO₃ (2 x 25 ml). The combined organic extracts were washed with brine and then dried over Na₂SO₄, stripped of solvent *in vacuo*. and The residue was subjected to chromatography (EtOAc : Pet. Ether 3:1 - 1:0) to give *the title compound* (1.27 g, 3 mmol, quant. yield) as a colourless oil.

$\nu_{\max}/\text{cm}^{-1}$ 3323 2850-2954 1742 1625 1575 1526 1469 1438 1405 1311 1187 1121 1032

δ_{P} (162 MHz, CDCl₃) 36.84* 37.14 (* denotes the distinguishable signals due to the major isomer)

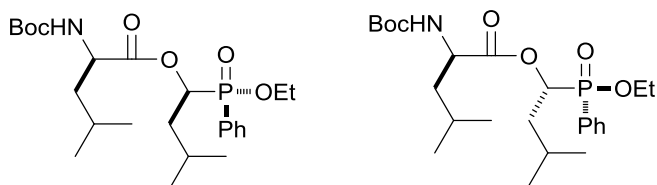
δ_{H} (400 MHz, CDCl₃) 0.82 (3H, d, *J* 6.1, CH₃), 0.86-0.91 (18H, m, 6x CH₃), 1.31-1.34 (6H, m, 2x OCH₂CH₃), 1.48-1.58 (4H, m, 2x NH₂CHCHCH₂), 1.65-1.69 (2H, m, 2x NH₂CHCH₂CHCHMe₂), 1.70-1.82 (6H, m, 2x PCHCH₂CHCHMe₂+ 2x PCHCHCH₂), 3.34 (1H, dd, *J* 5.6, 8.9, NH₂CHCH), 3.40 (1H, dd, *J* 5.1, 9.3, NH₂CHCH*), 3.97-4.04 (2H, m, OCH2CH₃), 4.10-4.18 (2H, m, OCH2CH₃*), 5.44 (1H, ddd, *J* 2.4, 4.0, *J*_P 11.5, PCHCH), 5.47 (1H, ddd, *J* 2.4, 4.4, *J*_P 11.0, PCHCH), 7.46-7.49 (4H, m, Ar H), 7.55-7.57 (2H, m, Ar H), 7.75-7.79 (4H, m, Ar H).

δ_C (101 MHz, $CDCl_3$) 16.70 (d, J_P 5.8, OCH_2CH_3), 16.71 (d, J_P 5.7, OCH_2CH_3), 21.20 (CH_3), 21.26 (CH_3), 21.58 (CH_3), 21.85 (CH_3), 23.08 (CH_3), 23.29 (CH_3), 23.35 (CH_3), 23.39 (CH_3), 24.61 ($CHMe_2$), 24.62 ($CHMe_2$), 24.69 ($CHMe_2$), 24.79 ($CHMe_2$), 37.20 (CH_2), 37.47 (CH_2), 43.46 (CH_2), 43.63 (CH_2), 52.98 (NH_2CH), 53.08 (NH_2CH), 61.70 (d, J_P 6.6, OCH_2CH_3), 61.71 (d, J_P 6.7, OCH_2CH_3), 69.05 (d, J_P 118.6, PCH), 69.20 (d, J_P 118.6, PCH), 128.48 (d, J_P 125.6, Ar C), 128.52 (d, J_P 125.1, Ar C), 128.76 (d, J_P 12.5, Ar CH), 128.82 (d, J_P 12.6, Ar CH), 132.50 (d, J_P 10.1, Ar CH), 132.56 (d, J_P 9.9, Ar CH), 133.01 (d, J_P 2.7, 2x Ar CH), 172.35 (d, J_P 3.1, CCO_2), 172.71 (d, J_P 3.4, CCO_2).

LRMS m/z (ESI) 370.4 $[M+H]^+$.

HRMS (ESI) calcd. for $C_{19}H_{33}O_4NP$ 370.2142, found 370.2140 $[M+H]^+$.

(R)-2-tert-Butoxycarbonylamino-4-methyl-pentanoic acid (S)-1-(ethoxy-phenyl-phosphinoyl)-3-methyl-butyl ester 392



DCC (4.15 g, 20 mmol), followed by DMAP (0.25 g, 2 mmol) were added to a stirred solution of compound **347** (1.03 g, 4.03 mmol) and Boc-(D)-Leu-OH (1.4 g, 6 mmol) in dichloromethane (100 ml) maintained at 0 °C. The mixture was left stirring for 3 h and the reaction monitored by TLC. Once complete (TLC) the reaction mixture was filtered to remove DHU and then stripped of solvent *in vacuo*. The residue was diluted with dichloromethane and poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na_2SO_4 , stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 4:1 - 1:1) to give *the title compound* (1.38 g, 3 mmol, 73% yield) as a colourless oil.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3321 2851-2957 1738 1706 1625 1574 1529 1469 1450 1438 1389 1365 1310
1281 1254 1221 1201 1161 1119 1095 1045 1018

δ_{P} (162 MHz, CDCl_3) 36.87*, 37.16

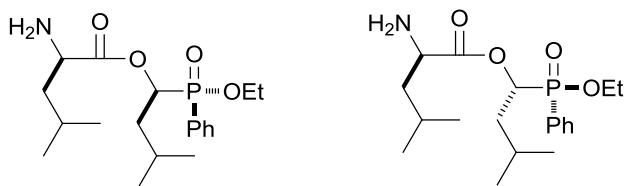
δ_{H} (400 MHz, CDCl_3) 0.77 (3H, d, J 6.4, CH_3), 0.79 (3H, d, J 7.7, CH_3), 0.83 (3H, d, J 3.4, CH_3), 0.84 (3H, d, J 5.3, CH_3), 0.88 (6H, d, J 6.5, 2x CH_3), 0.89 (6H, d, J 8.0, 2x CH_3), 1.28-1.32 (6H, m, 2x OCH_2CH_3), 1.38 [9H, s, $\text{C}(\text{CH}_3)_3$], 1.39 [9H, s, $\text{C}(\text{CH}_3)_3$], 1.41-1.52 (6H, m, 2x NHCHCH_2 + 2x $\text{NHCHCH}_2\text{CHMe}_2$), 1.57-1.67 (2H, m, 2x $\text{PCHCH}_2\text{CHMe}_2$), 1.70-1.88 (4H, m, 2x PCHCH_2), 3.93-4.03 (2H, m, OCH_2CH_3), 4.08-4.14 (2H, m, $\text{OCH}_2\text{CH}_3^*$), 4.23 (1H, td, J 4.7, 9.5, NHCH), 4.28 (1H, td, J 5.2, 9.2, NHCH^*), 4.78 (1H, d, J 8.9, NH^*), 4.83 (1H, d, J 8.9, NH), 5.39 (1H, d, J 11.3, PCH^*), 5.44 (1H, d, J 11.4, PCH), 7.44-7.46 (4H, m, Ar H), 7.51-7.55 (2H, m, Ar H), 7.72-7.77 (4H, m, Ar H).

δ_{C} (101 MHz, CDCl_3) 16.61 (d, J_{P} 6.2, OCH_2CH_3), 16.65 (d, J_{P} 6.0, OCH_2CH_3), 21.01 (CH_3), 21.11 (CH_3), 21.62 (CH_3), 21.74 (CH_3), 23.02 (CH_3), 23.16 (CH_3), 23.37 (CH_3), 23.38 (CH_3), 24.32 (CHMe_2), 24.40 (CHMe_2), 24.72 (CHMe_2), 24.80 (CHMe_2), 28.35 [$\text{C}(\text{CH}_3)_3$], 28.38 [$\text{C}(\text{CH}_3)_3$], 37.02 (CH_2^*), 37.40 (CH_2), 41.60 (CH_2), 41.78 (CH_2^*), 52.12 (NHCH), 52.31 (NHCH maj), 61.69 (d, J_{P} 6.5, OCH_2CH_3), 61.71 (d, J_{P} 6.7, OCH_2CH_3), 69.55 (d, J_{P} 118.9, 2x PCH), 79.73 (OCMe_3^*), 79.87 (OCMe_3), 128.23 (d, J_{P} 125.8, Ar C), 128.38 (d, J_{P} 126.1, Ar C), 128.81 (d, J_{P} 12.5, Ar CH), 128.86 (d, J_{P} 12.5, Ar CH*), 132.45 (d, J_{P} 9.7, Ar CH*), 132.47 (d, J_{P} 9.8, Ar CH), 132.94 (d, J_{P} 2.8, Ar CH*), 132.99 (d, J_{P} 2.6, Ar CH), 155.21 (NCO_2^*), 155.43 (NCO_2), 172.33 (d, J_{P} 3.7, CCO_2), 172.69 (d, J_{P} 4.03, CCO_2).

LRMS m/z (ESI) 487.4 $[\text{M}+\text{NH}_4]^+$, 470.3 $[\text{M}+\text{H}]^+$.

HRMS (ESI) calcd. for $\text{C}_{24}\text{H}_{41}\text{O}_6\text{N}_1\text{P}_1$ 470.2666, found 470.2667 $[\text{M}+\text{H}]^+$.

(R)-2-Amino-4-methyl-pentanoic acid (S)-1-(ethoxy-phenyl-phosphinoyl)-3-methyl-butyl ester 393



TFA (5 ml, 65 mmol) was added to a stirred solution of compound **392** (1.38 g, 2.94 mmol) in dichloromethane (75 ml) maintained at 0 °C for 1 h. On completion (TLC) the reaction mixture was poured into saturated NaHCO₃ (50 ml) and the organic layer was separated. The organic phase was washed with NaHCO₃ (2 x 25 ml). The combined organic extracts were washed with brine and then dried over Na₂SO₄, stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 3:1 - 1:0) to give *the title compound* (1.09 g, 3 mmol, quant. yield) as a colourless oil.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3325 2840-2960 1740 1620 1570 1520 1440 1410 1306 1183 1120 1030

δ_{P} (162 MHz, CDCl₃) 36.83*, 37.02

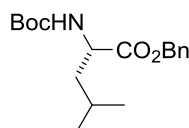
δ_{H} (400 MHz, CDCl₃) 0.84 (6H, d, *J* 5.8, 2x CH₃), 0.87-0.92 (18H, m, 6x CH₃), 1.28-1.32 (6H, m, 2x OCH₂CH₃), 1.23-1.28 (2H, m, NH₂CHCH₂), 1.29-1.32 (2H, m, NH₂CHCH₂), 1.34 (6H, tdd, *J* 1.7, 5.4, 7.0, 2x OCH₂CH₃), 1.44-1.50 (2H, m, 2x NH₂CHCH₂CHMe₂), 1.51-1.60 (2H, m, 2x PCHCH₂CHMe₂), 1.70-1.83 (4H, m, 2x PCHCH₂), 3.36 (1H, ddd, *J* 1.1, 5.6, 8.8, NH₂CH), 3.41 (1H, ddd, *J* 1.1, 5.1, 9.3, NH₂CH*), 3.98-4.06 (2H, m, OCH₂CH₃), 4.12-4.20 (2H, m, OCH₂CH₃*), 5.47 (1H, ddd, *J* 1.8, 4.0, *J*_P 12.1, PCH), 5.47 (1H, dddd, *J* 1.7, 2.5, 4.1, *J*_P 32.8, PCH), 7.47-7.50 (4H, m, Ar H), 7.56-7.59 (2H, m, Ar H), 7.76-7.80 (4H, m, Ar H).

δ_{C} (101 MHz, CDCl₃) 16.69 (d, *J*_P 5.9, OCH₂CH₃), 16.72 (d, *J*_P 5.8, OCH₂CH₃), 21.20 (CH₃), 21.26 (CH₃), 21.58 (CH₃), 21.86 (CH₃), 23.09 (CH₃), 23.30 (CH₃), 23.37 (CH₃),

23.40 (CH₃), 24.55 (CHMe₂), 24.63 (CHMe₂), 24.69 (CHMe₂), 24.79 (CHMe₂), 37.19 (CH₂), 37.46 (CH₂), 43.47 (CH₂), 43.65 (CH₂), 52.98 (NH₂CH), 53.09 (NH₂CH), 61.70 (d, *J*_P 6.7, OCH₂CH₃), 61.71 (d, *J*_P 6.7, OCH₂CH₃), 69.04 (d, *J*_P 118.6, PCH), 69.19 (d, *J*_P 118.4, PCH), 128.77 (d, *J*_P 125.7, Ar C), 128.50 (d, *J*_P 125.8, Ar C), 128.77 (d, *J*_P 12.4, Ar CH), 128.82 (d, *J*_P 12.6, Ar CH), 132.50 (d, *J*_P 9.9, Ar CH), 132.57 (d, *J*_P 10.1, Ar CH), 133.01 (d, *J*_P 2.8, 2x Ar CH), 175.51 (d, *J*_P 3.1, C=O), 175.73 (d, *J*_P 3.4, C=O).

LRMS *m/z* (ESI) 370.4 [M+H]⁺.

Boc-(S)-Leu-OBn 397



Caesium carbonate (14.8 g, 45 mmol) was added to a stirred solution of Boc-(L)-Leu-OH (10.5 g, 45 mmol) in DMF and the resulting solution was heated to 60 °C for 1 h. The reaction was cooled to 0 °C, benzyl bromide (5.4 ml, 45 mmol) was added slowly and the reaction mixture was again heated at 60 °C for a further 3 h. The solvent was removed *in vacuo* and the residue was diluted with dichloromethane and poured into water (50 ml) and the mixture was extracted with dichloromethane (2 x 25 ml). The combined organic extracts were washed with saturated NaHCO₃ then dried over Na₂SO₄ and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 8:1 - 4:1) to give the title compound (13.9 g, 43 mmol, 95% yield) as a colourless oil.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3362, 2871-2959, 1711, 1499, 1455, 1389, 1366, 1249, 1157, 1047, 1019.

δ_{H} (600 MHz, CDCl₃) 0.91 (3H, d, *J* 6.3, CH₃), 0.91 (3H, d, *J* 6.3, CH₃), 1.42 [9H, s, [C(CH₃)₃], 1.45-1.50 (2H, m, CH₂), 1.65-1.68 (1H, m, CHMe₂), 4.33-4.36 (1H, m,

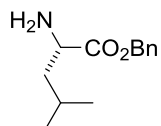
NHCHCO₂), 4.90 (1H, d, *J* 8.1, NH), 5.11-5.19 (2H, 2 x d, *J* 12.3, CH₂Ph), 7.31-7.36 (5H, m, Ar H).

δ_C (151 MHz, CDCl₃) 22.95 (2 x CH₃), 24.88 (CHMe₂), 28.43 [C(CH₃)₃], 41.82 (CH₂), 52.30 (CHCO₂), 67.01 (CH₂Ph), 79.96 (OCMe₃), 128.29 (2 x Ar CH), 128.49 (Ar CH), 128.68 (2 x Ar CH), 135.62 (Ar C), 155.54 (NCO₂), 173.51 (CCO₂).

LRMS *m/z* (ESI) 339.1 [M+NH₄]⁺, 322.1 [M+H]⁺.

HRMS (ESI) calcd. for C₁₈H₃₁O₄N₂ 339.2278, found 339.2285 [M+NH₄]⁺.

H₂N-(S)-Leu-OBn **398**



TFA (10 ml, 131 mmol) was added to a stirred solution of compound **397** (9.0 g, 28 mmol) in dichloromethane (100 ml) maintained at 0 °C and the solution was stirred for 1 h. On completion (TLC), the reaction mixture was poured into saturated NaHCO₃ (50 ml) and the organic layer was separated. The organic phase was washed with NaHCO₃ (2 x 25 ml). The combined organic extracts were washed with brine and then dried over Na₂SO₄ and stripped of solvent *in vacuo* to give the title compound (5.95 g, 27 mmol, 96% yield) colourless oil.

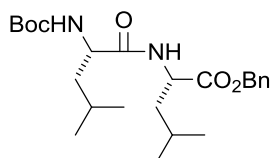
*v*_{max}/cm⁻¹ 3039 2870-2956 1743 1665 1609 1581 1515 1454 1406 1386 1367 1345 1322
1297 1255 1188 1142 1090 1033 1003

δ_H (400 MHz, CDCl₃) 0.93 (3H, d, *J* 6.7, CH₃), 0.94 (3H, d, *J* 6.7, CH₃), 1.56-1.59 (2H, m, CH₂), 1.60-1.65 (1H, m, CHMe₂), 1.84-1.92 (1H, m, CHCH₂), 5.21 (2H, d, *J* 6.0, CH₂Ph), 7.34-7.40 (5H, m, Ar H).

δ_C (101 MHz, $CDCl_3$) 17.71 (CH_3), 19.45 (CH_3), 20.59 ($CHMe_2$), 37.44 ($CHCO_2$), 39.58 (CH_2), 63.48 (CH_2Ph), 124.50 (Ar CH), 124.57 (Ar CH), 124.73 (Ar CH), 124.84 (Ar CH), 124.90 (Ar CH), 131.37 (Ar C), 171.94 (CCO_2).

LRMS m/z (ESI) 222.1 $[M+H]^+$

Boc-(S)-Leu-(S)-Leu-OBn 399



DCC (7.92 g, 38 mmol), followed by DMAP (0.47 g, 4 mmol) were added to a stirred solution of compound **398** (6.34 g, 35 mmol) and Boc-(L)-Leu-OH (8.88 g, 38 mmol) in dichloromethane (100 ml) maintained at 0 °C. The mixture was left stirring for 3 h and the reaction monitored by TLC. The reaction mixture was filtered to remove DHU and then stripped of solvent *in vacuo*. The residue was diluted with dichloromethane and poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na_2SO_4 and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 4:1 - 2:1) to give the title compound (13.47 g, 31 mmol, 89% yield) as a white solid.

mp 92-95 °C;

ν_{max}/cm^{-1} 3337 2870-2957 1727 1681 1661 1518 1454 1389 1366 1322 1292 1271 1248
1208 1163 1045 1022

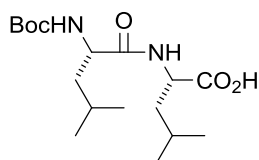
δ_H (600 MHz, $CDCl_3$) 0.89 (12H, d, J 4.4, CH_3), 1.42 [9H, s, $C(CH_3)_3$], 1.43-1.45 (2H, m, CH_2), 1.61-1.65 (4H, m, CH_2 + 2x $CHMe_2$), 4.08-4.10 (1H, m, $CHCH_2$), 4.62-4.66 (1H, m, $CHCH_2$), 5.14 (2H, 2x d, J 12.0, CH_2Ph), 7.31-7.36 (5H, m, Ar H).

δ_{C} (151 MHz, CDCl_3) 21.89 (CH_3), 21.97 (CH_3), 22.25 (CH_3), 22.97 (CH_3), 24.76 (CHMe_2), 24.81 (CHMe_2), 28.38 [$\text{C}(\text{CH}_3)_3$], 40.96 (CH_2), 41.55 (CH_2), 50.85 (NCHCO), 53.03 (CHCO_2), 67.16 (CH_2Ar), 80.16 (OCMe_3), 128.36 (Ar CH), 128.52 (Ar CH), 128.71 (Ar CH), 135.47 (Ar C), 155.82 (NCO_2), 172.30 (CON), 172.63 (CCO_2).

LRMS m/z (ESI) 452.1 $[\text{M}+\text{NH}_4]^+$.

HRMS (ESI) calcd. for $\text{C}_{24}\text{H}_{42}\text{O}_5\text{N}_3$ 452.3119, found 452.3116 $[\text{M}+\text{NH}_4]^+$.

Boc-(S)-Leu-(S)-Leu-OH **400**



A mixture of compound **399** (10.82 g, 25 mmol) and 5% Pd/C (2.16 g, 20% wt) in EtOAc (150ml) was stirred under a hydrogen atmosphere overnight. The catalyst was filtered through celite and the solvent was removed *in vacuo* to give the title compound (8.37 g, 24 mmol, 98% yield) as a white solid.

mp 150-153 °C; lit 153-155 °C

$\nu_{\text{max}}/\text{cm}^{-1}$ 3310 2872-2959 1659 1517 1452 1390 1367 1249 1160 1123 1047 1021

δ_{H} (400 MHz, CDCl_3) 0.88-0.93 (12H, m, CH_3), 1.40 [9H, s, $\text{C}(\text{CH}_3)_3$], 1.45-1.55 (2H, m, CH_2), 1.52-1.61 (2H, m, CH_2), 1.63-1.67 (2H, m, 2x CHMe_2), 4.15-4.20 (1H, m, NCHCO), 4.56-4.60 (1H, m, CHCO_2), 5.32 (1H, d, J 7.5, NH), 6.95 (1H, d, J 5.9, NH), 9.24 (1H, br s, CO_2H).

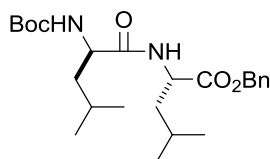
δ_{C} (101 MHz, CDCl_3) 21.80 (CH_3), 22.31 (CH_3), 22.82 (CH_3), 23.05 (CH_3), 24.71 (CHMe_2), 24.83 (CHMe_2), 28.36 [$\text{C}(\text{CH}_3)_3$], 40.82 (CH_2), 41.33 (CH_2), 50.93

(NCHCO), 53.12 (CHCO₂), 80.50 (OCMe₃), 156.21 (NCO₂), 173.11 (CON), 176.36 (CCO₂).

LRMS m/z (ESI) 343.2 [M+H]⁺.

HRMS (ESI) calcd. for C₁₇H₃₂O₅N₂Na 367.2203, found 367.2203 [M+Na]⁺.

Boc-(R)-Leu-(S)-Leu-OBn 401



DCC (2.81 g, 14 mmol), followed by DMAP (0.17 g, 1 mmol) were added to a stirred solution of compound **398** (1.51 g, 7 mmol) and Boc-(D)-Leu-OH (1.64 g, 7 mmol) in dichloromethane (100 ml) maintained at 0 °C. The mixture was left stirring for 3 h and the reaction monitored by TLC. Once complete (TLC) the reaction mixture was filtered to remove DHU and then stripped of solvent *in vacuo*. The residue was diluted with dichloromethane and poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na₂SO₄ and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 4:1 - 1:1) to give the title compound (2.61 g, 6 mmol, 88% yield) as a white solid.

mp 88-91 °C.

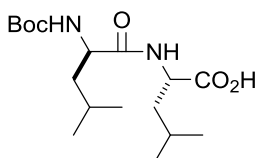
$\nu_{\max}/\text{cm}^{-1}$ 3320 2868-2951 1722 1686 1651 1524 1469 1456 1385 1366 1348 1320 1292
1274 1251 1237 1207 1165 1124 1047 1025

δ_{H} (400 MHz, CDCl₃) 0.90-0.96 (12H, m, 4x CH₃), 1.46 [9H, s, C(CH₃)₃], 1.55-1.69 (6H, m, 2x CHMe₂ + 2x CH₂), 4.15 (1H, m, NCHCO), 4.66 (1H, m, CHCO₂), 4.81 (1H, br s, NH), 5.17 (2H, q, *J* 12.3, CH₂Ph), 6.59 (1H, br s, NH), 7.33-7.41 (5H, m, Ar H).

δ_{C} (101 MHz, CDCl_3) 21.82 (CH_3), 22.85 (2x CH_3), 23.00 (CH_3), 24.80 (CHMe_2), 25.45 (CHMe_2), 28.25 [$\text{C}(\text{CH}_3)_3$], 41.37 (2x CH_2), 50.73 (CHCO_2), 53.08 (CHCO_2), 67.03 (CH_3), 80.22 (OCMe_3), 128.23 (Ar CH), 128.40 (Ar CH), 128.60 (Ar CH), 135.38 (Ar C), 139.90 (NCO_2), 172.65 ($\text{CON} + \text{CCO}_2$).

LRMS m/z (ESI) 435.2 $[\text{M}+\text{H}]^+$.

Boc-(R)-Leu-(S)-Leu-OH 402



A mixture of compound **401** (3.13 g, 7 mmol) and 5% Pd/C (0.63 g, 20 % wt) in EtOAc was stirred under a hydrogen atmosphere overnight. The catalyst was filtered through celite and the solvent was removed *in vacuo* to give the title compound (2.48 g, 7 mmol, quant. yield) as a white solid.

mp 154-157 °C.

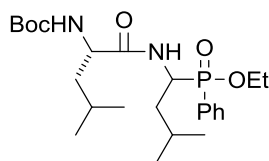
$\nu_{\text{max}}/\text{cm}^{-1}$ 3301 2870-2957 1697 1657 1521 1453 1388 1366 1248 1158 1046 1022

δ_{H} (400 MHz, CDCl_3) 0.94 (6H, d, J 6.3, 2x CH_3), 0.98 (6H, d, J 6.4, 2x CH_3), 1.46 [9H, s, $\text{C}(\text{CH}_3)_3$], 1.58-1.62 (2H, m, CH_2), 1.76-1.84 (4H, m, 2x $\text{CHMe}_2 + \text{CH}_2$), 3.95-3.97 (2H, m, $\text{NCHCO} + \text{CHCO}_2$).

δ_{C} (101 MHz, CDCl_3) 21.27 (2x CH_3), 23.41 (2x CH_3), 24.35 (2x CHMe_2), 28.38 [$\text{C}(\text{CH}_3)_3$], 43.69 (2x CH_2), 53.44 ($\text{NCHCO} + \text{CHCO}_2$), 80.22 (OCMe_3), 155.50 (NCO_2), 169.19 ($\text{CON} + \text{CCO}_2$).

LRMS m/z (ESI) 343.2 $[\text{M}+\text{H}]^+$.

[1-((S)-2-tert-Butoxycarbonylamino-4-methyl-pentanoylamino)-3-methyl-butyl]-phenyl-phosphinic acid ethyl ester 405



Triethylamine (0.7 ml, 4.7 mmol) was added to a stirred solution of Boc-(L)-Leu-OH (0.73 g, 3.1 mmol) and HBTU (1.4 g, 3.8 mmol) in THF (80 ml) maintained at 0 °C. After 30 min compound **354** (0.8 g, 3.14 mmol) was added and the mixture was left stirring for 3 h and the reaction monitored by TLC. Once complete the reaction mixture was filtered and then stripped of solvent *in vacuo*. The residue was diluted with dichloromethane and poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na₂SO₄ and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 4:1 - 1:1) to give *the title compound* (1.35 g, 2.9 mmol, 95% yield) as a colourless oil.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3279 3211 3054 2867-2957 1730 1706 1681 1657 1517 1467 1438 1388 1364
1299 1275 1245 1225 1198 1164 1121 1097 1014

δ_{P} (162 MHz, CDCl₃) (1.6:1) 40.65*, 40.76

δ_{H} (400 MHz, CDCl₃) 0.65-0.72 (6H, m, CH₃), 0.88-0.91 (6H, m, CH₃), 1.07-1.08 (2H, m, 2x CHMe₂), 1.20 (3H, t, *J* 7.1, OCH₂CH₃), 1.32 [9H, s, C(CH₃)₃], 1.64-1.72 (4H, m, CH₂ⁱPr), 3.73-3.86 (2H, m, NCHCO + OCH₂CH₃), 3.95-4.07 (1H, m, OCH₂CH₃), 4.62-4.72 (1H, m, PCH), 6.38 (1H, d, *J* 9.3, NH), 7.36-7.41 (2H, m, Ar H), 7.44-7.49 (1H, m, Ar H), 7.71-7.77 (2H, m, Ar H).

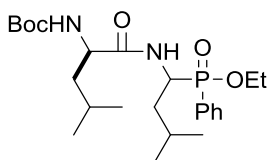
δ_{C} (101 MHz, CDCl₃) 16.37 (d, *J*_P 6.2, OCH₂CH₃), 16.41 (d, *J*_P 6.0, OCH₂CH₃), 21.21 (CH₃), 22.78 (CH₃), 23.46 (CH₃), 23.55 (CH₃), 24.26 (CHMe₂), 24.31 (CHMe₂), 24.38

(CHMe₂), 24.59 (CHMe₂), 28.26 [C(CH₃)₃], 36.69 (CH₂), 36.93 (CH₂), 41.10 (CH₂), 41.12 (CH₂), 45.32 (d, *J*_P 116.8, PCH), 45.33 (d, *J*_P 118.8, PCH), 52.80 (NCH), 61.36 (d, *J*_P 7.3, OCH₂CH₃), 79.75 (OCMe₃), 128.14 (d, *J*_P 120.0, Ar C), 128.30 (d, *J*_P 12.1, Ar CH), 128.42 (d, *J*_P 12.0, Ar CH), 128.43 (d, *J*_P 123.2, Ar C), 132.40 (d, *J*_P 4.4, Ar CH), 132.42 (d, *J*_P 9.0, Ar CH), 132.52 (d, *J*_P 9.7, Ar CH), 132.59 (d, *J*_P 4.4, Ar CH), 172.01 (NCO₂), 172.06 (NCO₂).

LRMS *m/z* (ESI) 491.4 [M+Na]⁺, 469.4 [M+H]⁺.

HRMS (ESI) calcd. for C₂₄H₄₂O₅N₂P 469.2826, found 469.2830 [M+H]⁺.

[1-((R)-2-tert-Butoxycarbonylamino-4-methyl-pentanoylamino)-3-methyl-butyl]-phenyl-phosphinic acid ethyl ester 406



Triethylamine (0.7 ml, 4.7 mmol) was added to a stirred solution of Boc-(D)-Leu-OH (0.73 g, 3.1 mmol) and HBTU (1.4 g, 3.8 mmol) in THF (80 ml) maintained at 0 °C. After 30 min compound **354a** (0.8 g, 3.14 mmol) was added and the mixture was left stirring for 3 h and the reaction monitored by TLC. Once complete the reaction mixture was filtered and then stripped of solvent *in vacuo*. The residue was diluted with dichloromethane and poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na₂SO₄ and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 4:1 - 1:1) to give *the title compound* (1.31 g, 2.8 mmol, 92% yield) as a colourless oil.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3281 2867-3053 1729 1706 1681 1659 1516 1364 1245 1198 1164 1121 1014

δ_{P} (162 MHz, CDCl₃) (0.65:1) 41.29, 41.42* (* denotes the distinguishable signals due to the major isomer)

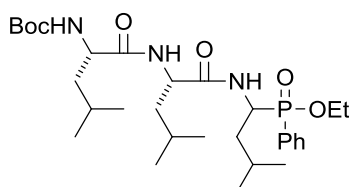
δ_{H} (400 MHz, CDCl_3) 0.67-0.74 (6H, m, CH_3), 0.86-0.93 (6H, m, CH_3), 1.24 (3H, t, J 7.2, OCH_2CH_3), 1.34 [9H, s, $\text{C}(\text{CH}_3)_3$], 1.37-1.40 (2H, m, 2x CHMe_2), 1.60-1.72 (4H, m, CH_2^{iPr}), 3.76-3.96 (2H, m, $\text{NCH} + \text{OCH}_2\text{CH}_3$), 3.95-4.07 (1H, m, OCH_2CH_3), 4.60-4.70 (1H, m, PCH), 7.01 (1H, d, J 10.0, NH), 7.38-7.46 (2H, m, Ar H), 7.49-7.52 (1H, m, Ar H), 7.70-7.76 (2H, m, Ar H).

δ_{C} (101 MHz, CDCl_3) 16.27 (d, J_{P} 6.2, OCH_2CH_3), 16.31 (d, J_{P} 6.1, OCH_2CH_3), 21.10 (CH_3), 22.75 (CH_3), 23.37 (CH_3), 23.47 (CH_3), 24.21 (CHMe_2), 24.31 (CHMe_2), 24.43 (CHMe_2), 24.49 (CHMe_2), 28.18 [$\text{C}(\text{CH}_3)_3$], 36.53 (CH_2), 36.72 (CH_2), 40.82 (CH_2), 41.12 (CH_2), 45.01 (d, J_{P} 116.7, PCH), 45.24 (d, J_{P} 116.7, PCH), 52.82 (NCH), 61.60 (d, J_{P} 7.1, OCH_2CH_3), 79.62 (OCMe_3), 128.39 (d, J_{P} 12.21, Ar CH), 128.51 (d, J_{P} 12.14, Ar CH), 129.31 (d, J_{P} 120.2, Ar C), 129.39 (d, J_{P} 118.7, Ar C), 132.25 (d, J_{P} 10.0, Ar CH), 132.38 (d, J_{P} 10.1, Ar CH), 132.79 (d, J_{P} 3.0, Ar CH), 132.84 (d, J_{P} 3.2, Ar CH), 172.49 (NCO_2), 172.92 (NCO_2).

LRMS m/z (ESI) 491.4 $[\text{M}+\text{Na}]^+$, 469.4 $[\text{M}+\text{H}]^+$.

HRMS (ESI) calcd. for $\text{C}_{24}\text{H}_{42}\text{O}_5\text{N}_2\text{P}$ 469.2826, found 469.2828 $[\text{M}+\text{H}]^+$.

{1-[(S)-2-((S)-2-tert-Butoxycarbonylamino-4-methyl-pentanoylamino)-4-methyl-pentanoylamino]-3-methyl-butyl}-phenyl-phosphinic acid ethyl ester 407



Triethylamine (0.24 ml, 2 mmol) was added to a stirred solution of compound **400** (0.57 g, 1.65 mmol) and HBTU (0.66 g, 1.7 mmol) in THF (100 ml) maintained at 0 °C. After 30 min compound **354a** (0.4 g, 2 mmol) was added and the mixture was left stirring for 3 h and the reaction monitored by TLC. Once complete the reaction mixture was filtered and then stripped of solvent *in vacuo*. The residue was diluted with

dichloromethane and poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na₂SO₄ and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 6:1 - 1:1) to give *the title compound* (0.82 g, 1 mmol, 90% yield) as a colourless oil.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3221 2850-2957 1689 1528 1439 1366 1208 1161 1121 1025

δ_{P} (162 MHz, CDCl₃) 40.98

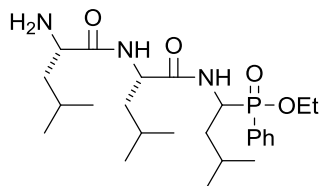
δ_{H} (400 MHz, CDCl₃) 0.70 (3H, d, *J* 6.5, βCH_3), 0.74 (3H, d, *J* 6.6, βCH_3), 0.86-0.87 (1H, m, γCHMe_2), 0.89 (3H, d, *J* 6.5, γCH_3), 0.90 (3H, d, *J* 6.6, γCH_3), 0.91 (3H, d, *J* 7.0, αCH_3), 0.93 (3H, d, *J* 6.6, αCH_3), 1.00-1.06 (1H, m, $\gamma\text{CH}_2^{\text{iPr}}$), 1.07-1.21 (1H, m, $\gamma\text{CH}_2^{\text{iPr}}$), 1.24 (3H, t, *J* 7.0, OCH₂CH₃), 1.31-1.35 (1H, m, βCHMe_2), 1.40-1.43 (2H, m, $\beta\text{CH}_2^{\text{iPr}}$), 1.45 [9H, s, C(CH₃)₃], 1.63-1.68 (1H, m, αCHMe_2), 1.74-1.82 (2H, m, $\alpha\text{CH}_2^{\text{iPr}}$), 3.80-3.87 (1H, m, OCH₂CH₃), 4.02-4.09 (1H, m, OCH₂CH₃), 4.13-4.18 (1H, m, βNHCH), 4.67 (1H, q, *J* 10.6, P αCH), 4.86 (1H, dd, *J* 7.3, 20.4, γNCHCO), 6.58 (1H, d, *J* 8.4, NH), 7.41-7.43 (2H, m, Ar H), 7.49-7.52 (1H, m, Ar H), 7.77-7.88 (2H, m, Ar H).

δ_{C} (101 MHz, CDCl₃) 16.52 (d, *J*_P 6.2, OCH₂CH₃), 21.22 (CH₃), 21.70 (CH₃), 22.97 (CH₃), 23.03 (CH₃), 23.57 (CH₃), 24.33 (CH₃), 24.42 (CHMe₂), 24.49 (CHMe₂), 24.88 (CHMe₂), 28.50 [C(CH₃)₃], 36.47 (CH₂), 40.65 (CH₂), 41.00 (CH₂), 45.36 (d, *J*_P 116.0, P αCH), 52.42 (βCHCO_2), 61.33 (d, *J*_P 7.2, OCH₂CH₃), 74.04 (γCHCO_2), 80.54 (OCMe₃), 128.29 (d, *J*_P 12.5, Ar CH), 128.45 (d, *J*_P 124.4, Ar C), 128.48 (d, *J*_P 124.1, Ar C), 128.53 (d, *J*_P 12.2, Ar CH), 132.08 (d, *J*_P 2.7, Ar CH), 132.41 (d, *J*_P 9.9, Ar CH), 132.54 (d, *J*_P 2.4, Ar CH), 132.71 (d, *J*_P 9.9, Ar CH), 155.93 (NCO₂), 169.71 (CCO), 172.08 (CCO).

LRMS *m/z* (ESI) 604.4 [M+Na]⁺, 582.5 [M+H]⁺.

HRMS (ESI) calcd. for C₃₀H₅₃O₆N₃P 582.3666, found 582.3670 [M+H]⁺.

{1-[(S)-2-((S)-2-Amino-4-methyl-pentanoylamino)-4-methyl-pentanoylamino]-3-methyl-butyl}-phenyl-phosphinic acid ethyl ester 408



TFA (2 ml, 26 mmol) was added to a stirred solution of compound **407** (0.18 g, 0.3 mmol) in dichloromethane (20 ml) maintained at 0 °C for 1 h. On completion of the reaction (TLC), the reaction mixture was poured into saturated NaHCO₃ (50 ml) and the organic layer was separated. The organic phase was washed with NaHCO₃ (2 x 25 ml). The combined organic extracts were washed with brine and then dried over Na₂SO₄ and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 4:1 - 1:0) to give *the title compound* (0.15 g, 0.31 mmol, quant. yield) as a colourless oil.

$\nu_{\max}/\text{cm}^{-1}$ 3217 2871-2957 1706 1666 1545 1469 1439 1387 1368 1207 1154 1122 1033

δ_{P} (162 MHz, CDCl₃) 41.04, 41.52.

δ_{H} (400 MHz, CDCl₃) 0.62-0.77 (6H, m, CH₃), 0.81-0.96 (12H, m, CH₃), 1.28 (3H, t, *J* 7.0, OCH₂CH₃), 1.41-1.48 (2H, m, CH₂ⁱPr), 1.56-1.76 (4H, m, 2x CH₂ⁱPr + CHMe₂), 1.95-2.08 (1H, m, CHMe₂), 3.69-3.81 (1H, m, OCH₂CH₃), 3.83-3.91 (1H, m, NH₂CH), 3.92-4.02 (1H, m, OCH₂CH₃), 4.39-4.51 (1H, m, NHCH), 4.62-4.71 (1H, m, PCH), 7.38-7.40 (2H, m, Ar H), 7.54-7.52 (1H, m, Ar H), 7.62-7.76 (2H, m, Ar H).

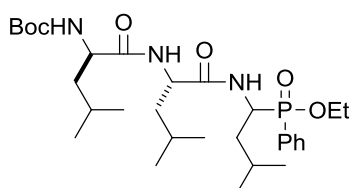
δ_{C} (101 MHz, CDCl₃) 16.39 (d, *J*_P 6.4, OCH₂CH₃), 21.09 (CH₃), 21.20 (CH₃), 21.32 (CH₃), 21.64 (CH₃), 23.14 (CH₃), 23.50 (CH₃), 25.00 (CHMe₂), 25.11 (CHMe₂), 25.85 (CHMe₂), 29.71 (CH₂), 34.23 (CH₂), 40.99 (CH₂), 45.24 (d, *J*_P 107.3, PCH), 51.19 (CHCO), 51.73 (CHCO), 61.63 (d, *J*_P 7.7, OCH₂CH₃), 126.33 (d, *J*_P 159.5, Ar C),

128.41 (d, J_p 13.3, Ar CH), 132.51 (d, J_p 10.3, Ar CH), 132.66 (d, J_p 2.7, Ar CH), 170.51 (CCO), 174.70 (CCO).

LRMS m/z (ESI) 504.4 $[M+Na]^+$, 482.4 $[M+H]^+$.

HRMS (ESI) calcd. for $C_{25}H_{44}N_3O_4P$ 482.3142, found 482.3146 $[M+H]^+$.

{1-[(S)-2-((R)-2-*tert*-Butoxycarbonylamino-4-methyl-pentanoylamino)-4-methyl-pentanoylamino]-3-methyl-butyl}-phenyl-phosphinic acid ethyl ester 409



Triethylamine (0.14 ml, 0.99 mmol) was added to a stirred solution of compound **402** (0.28 g, 0.82 mmol) and HBTU (0.33 g, 0.87 mmol) in THF (80 ml) maintained at 0 °C. After 30 min compound **354a** (0.2 g, 0.78 mmol) was added and the mixture was left stirring for 3 h and the reaction monitored by TLC. Once complete (TLC) the reaction mixture was filtered and then stripped of solvent *in vacuo*. The residue was diluted with dichloromethane and poured into water (50 ml) and the organic layer was extracted. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na_2SO_4 and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 6:1 - 2:1) to give *the title compound* (0.43 g, 0.74 mmol, 95% yield) as a colourless oil.

ν_{max}/cm^{-1} 3262 3060 2871-2957 1644 1527 1469 1439 1388 1366 1248 1210 1165 1122 1033

δ_P (162 MHz, $CDCl_3$) 40.98

δ_H (400 MHz, $CDCl_3$) 0.64 (3H, d, J 6.1, $^{\beta}CH_3$), 0.67 (3H, d, J 6.5, $^{\beta}CH_3$), 0.88-0.94 (12H, d, J 6.5, $^{\alpha,\gamma}CH_3$), 1.06-1.11 (1H, m, $^{\gamma}CHMe_2$), 1.23 (3H, t, J 6.9, OCH_2CH_3), 1.26-1.28 (1H, m, $^{\beta}CHMe_2$), 1.41 [9H, s, $C(CH_3)_3$], 1.47-1.49 (2H, m, $^{\gamma}CH_2^iPr$), 1.54-1.77

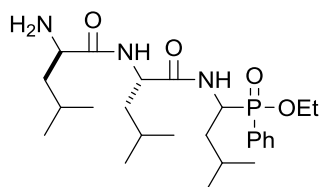
(5H, m, $^{\alpha}\text{CHMe}_2 + 2 \times ^{\alpha,\beta}\text{CH}_2^{\text{iPr}}$), 3.76-3.88 (1H, m, OCH_2CH_3), 3.99-4.11 (2H, m, J 7.0, $\text{OCH}_2\text{CH}_3 + ^{\gamma}\text{NHCH}$), 4.44-4.45 (0.5H, m, $^{\beta}\text{NHCH}$), 4.52-4.57 (0.5H, m, $^{\beta}\text{NHCH}$), 4.60-4.76 (1H, m, $\text{P}^{\alpha}\text{CH}$), 6.36 (0.5H, d, J 8.0, $^{\beta}\text{NH}$), 6.56 (0.5H, d, J 8.0, $^{\beta}\text{NH}$), 7.43-7.47 (2H, m, Ar H), 7.52-7.55 (1H, m, Ar H), 7.76-7.88 (2H, m, Ar H), 7.72 (0.5H, br s, NH a), 7.97 (0.5H, br s, NH a).

δ_{C} (101 MHz, CDCl_3) 16.34 (d, J_{P} 6.1, OCH_2CH_3), 16.36 (d, J_{P} 6.2, OCH_2CH_3), 21.12 (CH_3), 21.22 (CH_3), 21.28 (CH_3), 21.77 (CH_3), 22.10 (CH_3), 22.30 (CH_3), 22.64 (CH_3), 22.76 (CH_3), 22.81 (CH_3), 22.93 (CH_3), 23.44 (CH_3), 23.50 (CH_3), 24.31 (CHMe_2), 24.36 (CHMe_2), 24.42 (CHMe_2), 24.47 (CHMe_2), 24.63 (CHMe_2), 24.71 (CHMe_2), 28.31 [$\text{C}(\text{CH}_3)_3$], 36.54 (CH_2), 40.94 (CH_2), 41.66 (CH_2), 45.16 (d, J_{P} 117.3, $\text{P}^{\alpha}\text{CH}$), 50.87 ($^{\beta}\text{CHCO}$), 53.16 ($^{\gamma}\text{CHCO}$), 61.30 (d, J_{P} 6.8, OCH_2CH_3), 61.37 (d, J_{P} 6.5, OCH_2CH_3), 79.34 (OCMe_3), 79.82 (OCMe_3), 128.32 (d, J_{P} 11.9, Ar CH), 128.44 (d, J_{P} 11.8, Ar CH), 128.48 (d, J_{P} 4.0, Ar CH), 128.59 (d, J_{P} 124.2, Ar C), 132.38 (d, J_{P} 10.1, Ar CH), 132.56 (d, J_{P} 4.0, Ar CH), 132.59 (d, J_{P} 10.0, Ar CH), 155.82 (NCO_2), 171.82 (CCO), 172.33 (2x CCO), 172.74 (CCO).

LRMS m/z (ESI) 604.4 $[\text{M}+\text{Na}]^+$, 582.5 $[\text{M}+\text{H}]^+$.

HRMS (ESI) calcd. for $\text{C}_{30}\text{H}_{53}\text{O}_6\text{N}_3\text{P}$ 582.3666, found 582.3668 $[\text{M}+\text{H}]^+$.

{1-[(S)-2-((R)-2-Amino-4-methyl-pentanoylamino)-4-methyl-pentanoylamino]-3-methyl-butyl}-phenyl-phosphinic acid ethyl ester 410



TFA (2.5 ml, 33 mmol) was added to a stirred solution of compound **409** (1.07 g, 2 mmol) in dichloromethane (40 ml) maintained at 0 °C for 1 h. On completion of the reaction the reaction mixture was poured into saturated NaHCO_3 (50 ml) and the organic layer was separated. The organic phase was washed with NaHCO_3 (2 x 25 ml).

The combined organic extracts were washed with brine and then dried over Na₂SO₄ and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 4:1 - 1:0) to give *the title compound* (0.88 g, 12 mmol, 99% yield) as colourless crystals, a mixture of diastereomers.

mp 172-174 °C.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3271 3056 2868-2956 1674 1653 1641 1539 1469 1438 1385 1367 1255 1195
1121 1012

δ_{P} (162 MHz, CDCl₃) 40.19, 40.23.

δ_{H} (400 MHz, CDCl₃) 0.74 (3H, d, J 6.7, βCH_3), 0.77 (3H, d, J 6.3, βCH_3), 0.90 (3H, d, J 6.4, γCH_3), 0.92 (3H, d, J 6.4, γCH_3), 0.93 (3H, d, J 6.4, αCH_3), 0.97 (3H, d, J 7.8, αCH_3), 1.00-1.06 (1H, m, $\beta\text{CH}_2^{\text{iPr}}$), 1.10-1.20 (2H, m, βCHMe_2 + $\beta\text{CH}_2^{\text{iPr}}$), 1.24-1.32 (1H, m, γCHMe_2), 1.26 (3H, t, J 7.0, OCH₂CH₃), 1.59 (2H, ddd, J 4.5, 9.3, J_{P} 18.4 $\gamma\text{CH}_2^{\text{iPr}}$), 1.65-1.76 (3H, m, $\alpha\text{CH}_2^{\text{iPr}}$ + αCHMe_2), 3.32 (1H, dt, J 3.8, 9.7, $\gamma\text{NH}_2\text{CH}$), 3.81-3.91 (1H, m, OCH₂CH₃), 4.03-4.13 (1H, m, OCH₂CH₃), 4.27-4.35 (1H, m, βNHCH), 4.68-4.77 (1H, m, P αCH), 7.08-7.14 (1H, m, αNH), 7.38 (1H, t, J 8.4, βNH), 7.45-7.49 (2H, m, Ar H), 7.54-7.57 (1H, m, Ar H), 7.79-7.84 (2H, m, Ar H).

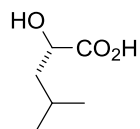
δ_{C} (101 MHz, CDCl₃) 16.38 (d, J_{P} 6.3, OCH₂CH₃), 21.18 (CH₃), 21.22 (CH₃), 21.30 (CH₃), 21.33 (CH₃), 21.83 (CH₃), 22.08 (CH₃), 22.89 (CH₃), 23.04 (CH₃), 23.40 (CH₃), 23.41 (CH₃), 23.47 (CH₃), 23.55 (CH₃), 24.42 (CHMe₂), 24.73 (CHMe₂), 24.82 (CHMe₂), 36.73 (CH₂), 36.84 (CH₂), 41.06 (CH₂), 41.44 (CH₂), 43.90 (CH₂), 44.00 (CH₂), 44.66 (d, J_{P} 116.4, P αCH), 45.28 (d, J_{P} 116.3, P αCH), 50.71 (βNHCH), 50.82 (βNHCH), 53.34 ($\gamma\text{NH}_2\text{CH}$), 53.51 ($\gamma\text{NH}_2\text{CH}$), 61.29 (d, J_{P} 7.1, OCH₂CH₃), 61.36 (d, J_{P} 6.9, OCH₂CH₃), 128.37 (d, J_{P} 12.5, Ar CH), 128.51 (d, J_{P} 123.9, Ar C), 132.51 (d,

J_P 10.2, Ar CH), 132.58 (d, J_P 3.5, Ar CH), 171.65 (d, J_P 4.3, CCO), 171.84 (d, J_P 4.2, CCO), 175.39 (CCO), 175.50 (CCO).

LRMS m/z (ESI) 504.4 $[M+Na]^+$, 482.4 $[M+H]^+$.

HRMS (ESI) calcd. for $C_{25}H_{44}N_3O_4P$ 482.3142, found 482.3148 $[M+H]^+$.

(S)-2-hydroxy-4-methylpentanoic acid 416¹¹³



A solution of sodium nitrite (31.0 g, 0.45 mol) in water (100 ml) was added dropwise over 3 h to a stirred solution of L-Leucine (9.84 g, 75 mmol) in 0.5M sulphuric acid (300 ml) maintained at 0 °C. Once the addition was complete the reaction was left to stir for 24 h at room temperature. The reaction mixture was extracted with diethyl ether (4 x 250 ml). The combined organic extracts were dried over Na₂SO₄ and stripped of solvent *in vacuo* to give a yellow oil which on standing crystallised to afford the title compound (7.52 g, 56.9 mmol, 76 % yield) as colourless crystals.

mp 79-81 °C. (lit. 79-80 °C)

$[\alpha]_D$ (c 1 g/ 100 ml) -2740.5° (lit., -2760.5)

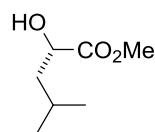
$\nu_{\max}/\text{cm}^{-1}$ 3419 2875-2932 1701 1468 1389 1362 1343 1268 1228 1170 1137 1120 1077

δ_H (600 MHz, CDCl₃) 0.95 (6H, d, J 6.0, 2 x CH₃), 1.57-1.65 (2H, m, CH₂), 1.86-1.93 (1H, m, CHMe₂), 4.27-4.29 (1H, dd, J 5.1, 8.4, CHCH₂).

δ_C (151 MHz, CDCl₃) 21.52 (CH₃), 23.31 (CH₃), 24.57 (CHMe₂), 43.29 (CH₂ⁱPr), 69.02 (CHCO₂H), 180.75 (CCO₂).

LRMS m/z (ESI) 131.07 $[M-H]^-$

(S)-2-Hydroxy-4-methylpentanoic acid methyl ester 417¹¹³



Acetyl chloride (0.30 ml, 4.16 mmol), was slowly added to a stirred solution of compound **416** (0.5 g, 3.8 mmol) in methanol (80 ml) maintained at 0 °C. Once the addition was complete the reaction was left to stir at room temperature for 1 h. After confirmation that the reaction was complete by TLC, the solution was concentrated in vacuo and poured into sat. NaHCO₃ to neutralise the acid. The reaction mixture was extracted with dichloromethane (2 x 25 ml). The combined extracts were dried over Na₂SO₄ and stripped of solvent *in vacuo* to give the title compound (396 mg, 72% yield) as a colourless oil.

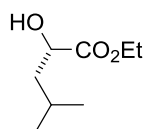
$\nu_{\text{max}}/\text{cm}^{-1}$ 3250 2890-2965 1670 1386 1224 1207 1165 1121

δ_{H} (400 MHz, CDCl₃) 0.93 (3H, d, *J* 6.7, CH₃), 0.98 (3H, d, *J* 6.7, CH₃), 1.55 (2H, m, CH₂), 1.86 (1H, m, CHMe₂), 3.76 (3H, s, OCH₃), 4.20 (1H, m, CHCO₂), 4.30 (1H, s, OH).

δ_{C} (101 MHz, CDCl₃) 21.92 (CH₃), 23.59 (CH₃), 24.60 (CHMe₂), 43.5 (CH₂), 52.6 (OCH₃), 69.1 (CHCO₂), 176.48 (CCO₂).

LRMS *m/z* (ESI) 147.0 [M+H]⁺

(S)-2-Hydroxy-4-methyl-pentanoic acid ethyl ester 418



Acetyl chloride (4.7 ml, 67 mmol), was added to a stirred solution of compound **416** (8.0 g, 61 mmol) in ethanol (100 ml) maintained at 0 °C. The solution was allowed to stir for 1 h and monitored by TLC. On completion of the reaction, the solvent was removed *in vacuo*. The reaction mixture was poured into water (50 ml) and was washed with dichloromethane (2 x 25 ml). The combined organic extracts were washed with saturated NaHCO₃, followed by brine and then dried over Na₂SO₄ and stripped of solvent *in vacuo* to give the title compound (9.07 g, 57 mmol, 93 % yield) as a colourless oil.

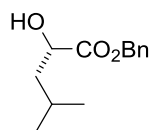
$\nu_{\text{max}}/\text{cm}^{-1}$ 3258 2899-2957 1672 1590 1437 1386 1224 1207 1162 1121 1099 1049 1018

δ_{H} (400 MHz, CDCl₃) 0.97 (6H, d, *J* 5.8, CH₃), 1.29 (3H, t, *J* 7.1, OCH₂CH₃), 1.59-1.69 (1H, m, CHMe₂), 1.86-1.96 (2H, m, CH₂ⁱPr), 4.25-4.32 (2H, m, OCH₂CH₃), 5.11-5.15 (1H, m, CHCO₂).

δ_{C} (101 MHz, CDCl₃) 14.10 (OCH₂CH₃), 21.43 (CH₃), 21.53 (CH₃), 28.96 (CHMe₂), 39.89 (CH₂), 52.71 (OCH), 61.46 (OCH₂CH₃), 170.18 (C=O).

LRMS *m/z* (ESI) 161.1 [M+H]⁺

(S)-2-Hydroxy-4-methyl-pentanoic acid benzyl ester **419**



NaHCO₃ (0.35 g, 4.2 mmol), was added to a stirred solution of compound **416** (0.50 g, 3.8 mmol) in DMF heated to 60 °C for 1 h. The reaction was cooled to 0 °C, benzyl bromide (0.5 ml, 4.2 mmol) was added slowly and the reaction mixture was heated at 60 °C for a further 3 h. The solvent was removed *in vacuo* and the residue was diluted with dichloromethane and poured into water (50 ml) and the organic layer was

separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were washed with saturated NaHCO₃ then dried over Na₂SO₄ and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 10:1 - 4:1) to give the title compound (0.67 g, 3 mmol, 80% yield) as a colourless oil.

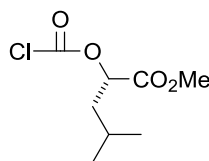
$\nu_{\text{max}}/\text{cm}^{-1}$ 3360 2860-2957 1731 1455 1368 1195 1137 1081

δ_{H} (400 MHz, CDCl₃) 0.85 (3H, d, *J* 6.7, CH₃), 0.86 (3H, d, *J* 6.7, CH₃), 1.48-1.52 (2H, m, CH₂), 1.79-1.88 (1H, m, CHMe₂), 4.18 (1H, dd, *J* 5.7, 7.6, CHⁱPr), 5.13 (2H, s, CH₂Ar), 7.25-7.31 (5H, m, Ar H).

δ_{C} (101 MHz, CDCl₃) 21.54 (CH₃), 23.22 (CH₃), 24.40 (CHMe₂), 43.36 (CH₂), 67.34 (CH₂Ar), 128.33 (Ar CH), 128.57 (Ar CH), 128.67 (Ar CH), 135.18 (Ar C), 175.83 (CCO₂).

LRMS *m/z* (ESI) 223.1 [M+H]⁺

***(S)*-methyl 2-((chlorocarbonyl)oxy)-4-methylpentanoate 420**



Triphosgene (0.295 g, 0.99 mmol), followed by pyridine (0.24 ml, 2.98 mmol) were slowly added to a solution of compound **417** (396 mg, 2.71 mmol) in diethyl ether (80 ml) and the resulting mixture was stirred for 3 h at 0 °C under a dry nitrogen atmosphere. Once the reaction was complete (TLC) the solution was concentrated in *vacuo*. The residue was poured into 0.1M HCl (60 ml) and the mixture was extracted with dichloromethane (2 x 50 ml). The organic extracts were washed with water and

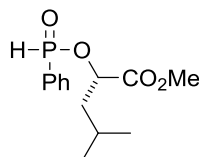
were dried over Na₂SO₄. The solvent was removed *in vacuo* to give the title compound as an oil (450 mg, 79% yield).

δ_{H} (400 MHz, CDCl₃) 0.74 (3H, d, *J* 6.5, CH₃), 0.81 (3H, d, *J* 6.5, CH₃), 1.68 (2H, m, CH₂), 1.75 (1H, m, CHMe₂), 5.18 (3H, s, OCH₃), 4.10 (1H, m, CHⁱPr).

δ_{C} (101 MHz, CDCl₃) 21.56 (CH₃), 22.46 (CH₃), 24.74 (CHMe₂), 39.32 (CH₂), 69.24 (CH), 53.67 (OCH₃), 149.9 (COCl), 167.91 (CCO₂).

LRMS *m/z* (ESI) 210.6 [M+H]⁺

(2S)-methyl 4-methyl-2-((phenylhydrophosphoryl)oxy)pentanoate 422



Phenylphosphinic acid (0.34 g, 2.38 mmol) was added to a solution of compound **420** (450 mg, 2.16 mmol) in dichloromethane (80 ml) followed by careful addition of pyridine (0.20 ml, 2.38 mmol) (Caution! Exothermic reaction; Rapid Effervescence) at 0 °C. The solution was removed from the ice bath and left to stir for 30 min. The reaction was then warmed to 40 °C and left to stir for 1 h. The solution was poured into 0.1M HCl (20 ml) and the organic layer was extracted with dichloromethane (2 x 50 ml). The organic extracts were washed with water and were dried using Na₂SO₄. The solvent was removed *in vacuo* to give the title compound as a colourless oil. (520 mg, 89% yield).

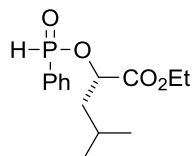
δ_{P} (162 MHz, CDCl₃) 45.86, 45.93.

δ_{H} (400 MHz, CDCl₃) 0.77 (3H, d, *J* 6.7, CH₃), 0.79 (3H, d, *J* 6.7, CH₃), 1.50 (2H, m, CH₂), 1.66 (1H, m, CHMe₂), 3.58 (3H, s, OCH₃), 4.83 (1H, m, CHⁱPr), 7.27-7.65 (5H, m, Ar H), 7.55 (1H, d, *J*_P 560.0, PH).

δ_C (101 MHz, $CDCl_3$) 21.42 (CH_3), 22.84 (CH_3), 24.37 ($CHMe_2$), 39.67 (CH_2), 52.11 (OCH_3), 74.42 (d, J_P 100.6, PCH), 128.42 (d, J_P 13.2, Ar CH), 130.19 (d, J_P 11.5, Ar CH), 131.55 (d, J_P 2.8, Ar CH), 135.79 (d, J_P 128.4, Ar C), 170.63 (CCO_2).

LRMS m/z (ESI) 271.3 $[M+H]^+$.

(2S)-ethyl 4-methyl-2-((phenylhydrophosphoryl)oxy)pentanoate **423**



Triphosgene (3.07 g, 10 mmol), followed by pyridine (2.5 ml, 31 mmol) were added to a stirred solution of compound **421** (4.52 g, 28 mmol) in dichloromethane (100 ml) maintained at 0 °C under a dry nitrogen atmosphere. The mixture was left stirring for approximately 3 h at 0 °C until formation of the chloroformate was complete. Pyridine (2.5 ml, 31 mmol) was slowly added to the stirred solution of the chloroformate and phenylphosphinic acid (4.41 g, 31 mmol). (Caution! Exothermic reaction; Rapid Effervescence). Once the effervescence had stopped the stirred solution was left to warm up to room temperature under a dry nitrogen atmosphere then refluxed for 1-2 h. Once the reaction was confirmed complete the solution was poured into 0.1M hydrochloric acid (50 ml) and the organic layer was separated. The organic extracts were washed with water (2 x 25 ml) and dried using Na_2SO_4 . The combined organic extracts were stripped of solvent *in vacuo* to give the title compound (7.60 g, 27 mmol, 95 % yield) as a colourless oil.

δ_P (162 MHz, $CDCl_3$) 26.05, 26.49*.

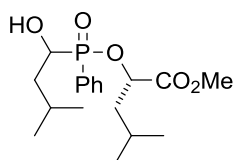
δ_H (400 MHz, $CDCl_3$) 0.87 (6H, d, J 6.7, 2x CH_3), 1.25 (3H, t, J 7.0, OCH_2CH_3), 1.50-1.54 (2H, m, CH_2), 1.83 (1H, sep, 6.7, $CHMe_2$), 4.02-4.08 (1H, m, OCH_2CH_3), 4.17-

4.21 (2H, m, $\underline{\text{CHCH}}_2 + \text{OCH}_2\underline{\text{CH}}_3$), 7.40-7.44 (2H, m, Ar H), 7.49-7.53 (1H, m, Ar H), 7.59 (1H, d, J_{PH} 561.2, P-H), 7.69-7.75 (2H, m, Ar H).

δ_{C} (101 MHz, CDCl_3) 16.8 (d, J_{P} 6.2, OCH_2CH_3), 21.46 (CH_3), 23.33 (CH_3), 24.43 (CH), 43.12 (CH_2), 62.35 (d, J_{P} 7.3, OCH_2CH_3), 68.82 (CH), 128.63 (d, J_{P} 13.8, 2x Ar H), 130.63 (d, J_{P} 12.0, 2x Ar H), 131.26 (Ar C), 132.58 (d, J_{P} 132.6, Ar H).

LRMS m/z (ESI) 285.1 $[\text{M}+\text{H}]^+$

(2S)-methyl 2-(((1-hydroxy-3-methylbutyl)(phenyl)phosphoryl)oxy)-4-methylpentanoate 426



Triethylamine (2.1 ml, 15.1 mmol) was added to a stirred solution of compound **422** (3.70 g, 13.7 mmol) in dichloromethane (150 ml) maintained at 0 °C under a dry nitrogen atmosphere. After 15 min TMSCl (1.9 ml, 15.1 mmol) was slowly added (Caution!: Excessive fuming) and the reaction mixture was left stirring for a further 15 min. Isovaleraldehyde (1.47 ml, 13.7 mmol) was added and the reaction mixture was left to warm up to room temperature overnight under a dry nitrogen atmosphere. Once the reaction was complete the reaction mixture was poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na_2SO_4 and stripped of solvent *in vacuo* to give crude compound **422** (5.55 g, 12.9 mmol, 95 % yield) as a colourless oil. Tetrabutylammonium fluoride (5 ml of a 1M solution in THF) was added to a stirred solution of compound **422** (5.55 g, 12.9 mmol) in diethyl ether (75 ml) / THF (75 ml). The reaction mixture was poured into water (50 ml) and was extracted with dichloromethane (3 x 25 ml). The combined organic extracts were dried over Na_2SO_4

and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 6:1 - 1:0) to give the title compound (4.59 g, 12.9 mmol, quant. yield) as a colourless oil. (see Appendix II)

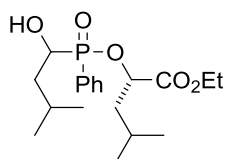
$\nu_{\max}/\text{cm}^{-1}$ 3260 2855-2965 1750 1580 1460 1430 1355 1270 1192 1119 1068

δ_{P} (162 MHz, CDCl_3) 41.87, 42.10, 42.27, 43.40 (1 : 1.7 : 2.5 : 1.6).

δ_{H} (400 MHz, CDCl_3) 0.72-0.93, 1.20-1.87, 3.16-5.10, 7.14-7.85.

LRMS m/z (ESI) 357.2 $[\text{M}+\text{H}]^+$

(2S)-ethyl 2-(((1-hydroxy-3-methylbutyl)(phenyl)phosphoryl)oxy)-4-methylpentanoate 427



Triethylamine (7.7 ml, 56 mmol) was added to a stirred solution of compound **423** (14.36 g, 51 mmol) in dichloromethane (150 ml) maintained at 0 °C under a dry nitrogen atmosphere. After 15 min TMSCl (7 ml, 56 mmol) was slowly added (Caution! Excessive fuming) and the reaction mixture was left stirring for a further 15 min. Isovaleraldehyde (6 ml, 56 mmol) was added and the reaction mixture was left to warm up to room temperature overnight under a dry nitrogen atmosphere. Once the reaction was complete the reaction mixture was poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na_2SO_4 and stripped of solvent *in vacuo* to give *crude compound* **425** (20.68 g, 47 mmol, 93% yield) as a colourless oil. Tetrabutylammonium fluoride (10 ml of a 1M solution in THF) was added to a stirred solution of compound **425** (20.68 g, 47 mmol) in diethyl ether (75 ml) / THF (75 ml).

The reaction mixture was poured into water (50 ml) and was extracted with dichloromethane (3 x 25 ml). The combined organic extracts were dried over Na₂SO₄ and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 4:1 - 1:1) to give the title compound as a colourless oil (17.32 g, 47 mmol, quant. yield), a mixture of diastereomers. (see Appendix II)

$\nu_{\text{max}}/\text{cm}^{-1}$ 3282 2870-2957 1754 1593 1468 1438 1369 1272 1193 1120 1068 1025

δ_{P} (162 MHz, CDCl₃) 41.97, 42.55, 42.59, 43.19 (ratio 1 : 0.42 : 0.56 : 0.63)

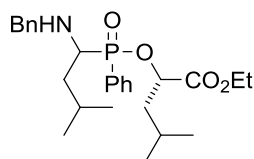
δ_{H} (400 MHz, CDCl₃) 0.67-0.77 (26H, m, CH₃), 0.80-0.87 (8H, m, CH₃), 0.89-0.93 (3H, m, OCH₂CH₃), 1.13-1.18 (6H, m, OCH₂CH₃), 1.20-1.88 (17H, m, CH + CH₂), 3.78-3.90 (2H, OCH₂CH₃), 3.93-4.02 (2H, CH), 4.04-4.16 (5H, m, CH + OCH₂CH₃), 4.61-4.66 (1H, m, CH), 4.72-4.77 (1H, m, CH), 4.89-4.94 (2H, m, CH), 7.27-7.33 (6H, m, Ar H), 7.36-7.41 (3H, m, Ar H), 7.70-7.78 (6H, m, Ar H).

δ_{C} (101 MHz, CDCl₃) 13.70 (OCH₂CH₃), 13.95 (OCH₂CH₃), 13.99 (OCH₂CH₃), 20.78 (CH₃), 20.86 (CH₃), 20.97 (CH₃), 21.02 (CH₃), 21.06 (CH₃), 21.10 (CH₃), 21.64 (CH₃), 21.66 (CH₃), 22.89 (CH), 22.93 (CH), 23.33 (CH), 23.42 (CH), 23.49 (CH), 23.81 (CH), 23.95 (CH), 24.02 (CH), 24.21 (CH), 38.45 (d, J_{P} 2.2, CH₂), 38.57 (d, J_{P} 3.9, CH₂), 39.01 (d, J_{P} 3.5, CH₂), 39.31 (d, J_{P} 3.4, CH₂), 41.85 (d, J_{P} 4.7, CH₂), 42.00 (d, J_{P} 4.7, CH₂), 42.22 (d, J_{P} 4.6, CH₂), 42.26 (d, J_{P} 4.4, CH₂), 60.96 (OCH₂CH₃), 61.40 (OCH₂CH₃), 61.83 (OCH₂CH₃), 67.18 (d, J_{P} 116.3, PCH), 68.00 (d, J_{P} 108.9, PCH), 68.47 (d, J_{P} 115.1, PCH), 69.09 (d, J_{P} 108.6, PCH), 70.94 (d, J_{P} 6.7, OCH), 71.65 (d, J_{P} 7.0, OCH), 71.72 (d, J_{P} 7.4, OCH), 71.79 (d, J_{P} 7.3, OCH), 127.96 (d, J_{P} 9.3, Ar CH), 128.04 (d, J_{P} 7.1, Ar CH), 128.11 (d, J_{P} 123.2, Ar C), 128.18 (d, J_{P} 117.6, Ar C), 128.19 (d, J_{P} 12.3, Ar CH), 128.28 (d, J_{P} 12.0, Ar CH), 128.40 (d, J_{P} 117.4, Ar C), 128.86 (d, J_{P} 121.6, Ar C), 131.79 (d, J_{P} 9.4, Ar CH), 131.96 (d, J_{P} 9.2, Ar CH), 132.42 (d, J_{P} 5.6,

Ar CH), 132.52 (d, J_P 5.5, Ar CH), 170.78 (d, J_P 2.8, CCO₂), 171.82 (d, J_P 1.9, CCO₂), 172.76 (d, J_P 1.6, CCO₂).

LRMS m/z (ESI) 372 [M+H]⁺

Ethyl 2-(((1-(benzylamino)-3-methylbutyl)(phenyl)phosphoryl)oxy)-4-methylpentanoate 431



Triethylamine (4.5 ml, 32 mmol) was added to a stirred solution of compound **423** (7.60 g, 27 mmol) in dichloromethane (150 ml) maintained at 0 °C under a dry nitrogen atmosphere. After 15 min TMSCl (4 ml, 32 mmol) was slowly added (Caution! Excessive fuming) and the reaction mixture was left stirring for a further 15 min. Imine **379** (6.06 g, 32 mmol) was added and the reaction mixture was left to warm up to room temperature overnight under a dry nitrogen atmosphere. Once the reaction was complete the reaction mixture was poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na₂SO₄ and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 8:1 - 1:1) to give the title compound (12.2 g, 27 mmol, quant. yield) as a colourless oil. (see Appendix II)

$\nu_{\max}/\text{cm}^{-1}$ 2869-2956 1751 1669 1592 1495 1453 1438 1386 1368 1270 121 1119 1068 1025

δ_P (162 MHz, CDCl₃) (2.13:1:1.61:3.59) 44.88, 45.04, 45.26, 45.48.

δ_H (400 MHz, CDCl₃) 0.50-0.94 (?H, m, CH₃, OCH₂CH₃), 1.06-1.16 (?H, m, OCH₂CH₃), 1.19-1.82 (?H, CH + CH₂), 2.94-3.06 (?H, m, PCH), 3.41-3.91 (?H, CH₂Ar

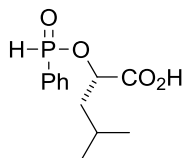
+ OCH₂CH₃), 3.97-4.13 (?H, m, OCH₂CH₃), 4.60-4.75 (?H, m, PCH), 6.87-7.76 (?H, m, Ar H).

δ_C (101 MHz, CDCl₃) 13.90 (OCH₂CH₃), 14.17 (OCH₂CH₃), 21.05 (CH₃), 21.19 (CH₃), 21.34 (CH₃), 21.44 (CH₃), 21.50 (CH₃), 21.95 (CH₃), 21.98 (CH₃), 22.07 (CH₃), 22.94 (CMe₂), 23.42 (CMe₂), 23.49 (CMe₂), 24.06 (CMe₂), 24.23 (CMe₂), 24.42 (CMe₂), 24.53 (CMe₂), 37.90 (d, J_P 2.6, CH₂), 38.00 (d, J_P 3.5, CH₂), 38.31 (d, J_P 5.2, CH₂), 42.21 (d, J_P 5.4, CH₂), 42.30 (d, J_P 5.0, CH₂), 42.66 (d, J_P 3.9, CH₂), 52.11 (d, J_P 3.9, CH₂Ar), 52.17 (d, J_P 4.8, CH₂Ar), 52.36 (d, J_P 2.8, CH₂Ar), 52.54 (d, J_P 4.3, CH₂Ar), 54.55 (d, J_P 134.3, PCH), 54.59 (d, J_P 134.6, PCH), 54.71 (d, J_P 101.5, PCH), 54.92 (d, J_P 107.3, PCH), 61.06 (OCH₂CH₃), 61.09 (OCH₂CH₃), 61.34 (OCH₂CH₃), 71.45 (d, J_P 7.5, PCH), 71.53 (d, J_P 7.6, PCH), 71.57 (d, J_P 7.7, PCH), 71.60 (d, J_P 7.7, PCH), 126.94 (Ar CH), 127.01 (Ar CH), 127.86-128.94 (Ar CH), 128.23 (Ar CH), 128.37 (Ar CH), 130.42 (d, J_P 115.9, Ar C), 130.20 (d, J_P 115.4, Ar C), 131.97-132.81 (Ar CH), 140.28 (Ar C), 140.40 (Ar C), 171.51 (CCO₂), 171.53 (CCO₂), 171.56 (CCO₂), 171.58 (CCO₂).

LRMS m/z (ESI) 460.4 [M+H]⁺.

HRMS (ESI) calcd. for C₂₆H₃₉O₄NP 460.2611, found 460.2608 [M+H]⁺.

(2S)-4-methyl-2-((phenylhydrophosphoryl)oxy)pentanoic acid 434



Triphosgene (2.52 g, 9 mmol), followed by pyridine (2 ml, 26 mmol) were added to a stirred solution of compound **416** (3.06 g, 23 mmol) in dichloromethane (150 ml) maintained at 0 °C under a dry nitrogen atmosphere. The mixture was left stirring for

approximately 3 h at 0 °C and compound **322** (3.62 g, 26 mmol) was added. Pyridine (2.1 ml, 26 mmol) was then slowly added to the stirred solution (Caution! Exothermic reaction; Rapid Effervescence). Once the effervescence had stopped the stirred solution was left to warm up to room temperature under a dry nitrogen atmosphere and was then refluxed for 1-2 h. Once the reaction was complete (TLC), the solution was poured into 0.1M hydrochloric acid (50 ml) and the organic layer was separated. The organic extracts were washed with water (2 x 25 ml) and dried over Na₂SO₄. The combined organic extracts were stripped of solvent *in vacuo* to give *the title compound* (5.93 g, 23 mmol, quant. yield) as a colourless oil.

$\nu_{\text{max}}/\text{cm}^{-1}$ 2870-2959, 2586, 2408, 2162, 1680, 1589, 1483, 1439, 1402, 1313, 1168, 1147, 1095, 1003.

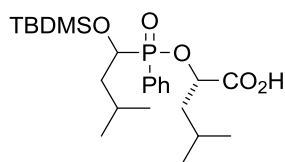
δ_{P} (162 MHz, CDCl₃) 27.80, 28.74.

δ_{H} (400 MHz, CDCl₃) 0.81 (3H, d, *J* 6.1, CH₃), 0.84 (3H, d, *J* 6.3, CH₃), 1.67-1.72 (3H, m, CHMe₂ + CH₂), 4.86-4.93 (1H, m, CHⁱPr), 7.40-7.49 (2H, m, Ar H), 7.53-7.58 (1H, m, Ar H), 7.70-7.81 (2H, m, Ar H), 7.71 (1H, d, *J*_P 577.3, PH).

δ_{C} (101 MHz, CDCl₃) 21.09 (CH₃), 23.09 (CH₃), 24.32 (CH), 41.23 (CH₂), 73.20 (d, *J*_P 7.6, CH), 74.52 (d, *J*_P 7.3, CH), 128.81 (d, *J*_P 137.0, Ar C), 128.87 (d, *J*_P 14.4, 2x Ar H), 128.93 (d, *J*_P 136.0, Ar C), 130.72 (d, *J*_P 12.7, Ar CH), 130.85 (d, *J*_P 12.9, Ar CH), 133.50 (d, *J*_P 2.9, Ar CH), 133.60 (d, *J*_P 2.8, Ar CH), 173.04 (CCO₂).

LRMS *m/z* (ESI) 257.1 [M+H]⁺.

(2S)-2-(((1-((tert-butyldimethylsilyl)oxy)-3-methylbutyl)(phenyl)phosphoryl)oxy)-4-methylpentanoic acid 437



TBDMSCl (2.12 g, 14 mmol) was added in small portions to a stirred solution of imidazole (0.96 g, 14 mmol) and compound **436** (3.21 g, 9 mmol) in dichloromethane (100ml) cooled to 0 °C for the addition. The reaction mixture was left to warm to rt and stirred for 5 h. Once the reaction was complete the solid was filtered and the remaining solvent removed *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 10:1 - 1:1) to give *the title compound* (4.28 g, 9 mmol, quant. yield) as a colourless oil. (see Appendix II)

$\nu_{\text{max}}/\text{cm}^{-1}$ 2858-2956 1740 1470 1439 1387 1368 1251 1173 1119 1064 1003

δ_{P} (162 MHz, CDCl_3) 42.98, 43.55, 44.39, (1 : 1.1 : 0.5)

δ_{H} (400 MHz, CDCl_3) -0.02, 0.00, 0.001 (18H, 3x s, $\text{C}(\text{CH}_3)_3\text{Si}(\text{CH}_3)_2$), 0.64-0.97 (36H, m, CH_3), 0.71, 0.75, 0.78 [27H, 3x s, $\text{C}(\text{CH}_3)_3\text{SiMe}_2$], 4.02-4.17 (2H, m, 2x PCH), 4.34 (1H, td, J 3.17, 9.89, PCH), 4.65-4.70, 4.76-4.85 (3H, m, CHCH_2), 7.30-7.35 (4H, m, Ar-H), 7.37-7.46 (4H, m, Ar-H), 7.48-7.51 (1H, m, Ar-H), 7.67-7.72 (1H, m, Ar-H), 7.74-7.79 (5H, m, Ar-H), 9.76 (3H, s, OH).

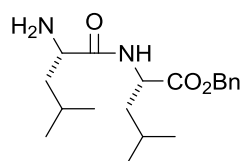
δ_{C} (101 MHz, CDCl_3) -5.22, -5.01, -4.86, -4.57, -4.45, -4.41, 18.17, 18.23, 21.42 (CH_3), 21.77 (CH_3), 21.84 (CH_3), 21.97 (CH_3), 22.93 (CH_3), 23.13 (CH_3), 23.42 (CH_3), 23.56 (CH_3), 23.64 (CH_3), 23.77 (CH_3), 23.88 (CH_3), 24.28 (CHMe_2), 24.32 (CHMe_2), 24.36 (CHMe_2), 25.78 ($[\text{C}(\text{CH}_3)_3]$), 25.81 ($[\text{C}(\text{CH}_3)_3]$), 25.85 ($[\text{C}(\text{CH}_3)_3]$), 41.25 (d, J_{P} 4.9, CH_2), 42.02 (d, J_{P} 5.0, CH_2), 42.30 (d, J_{P} 4.3, CH_2), 70.02 (d, J_{P} 121.7, PCH), 69.98 (d, J_{P} 120.8, PCH), 69.78 (d, J_{P} 121.6, PCH), 73.07 (d, J_{P} 8.2, CH), 73.04 (d, J_{P} 7.2, CH), 72.91 (d, J_{P} 8.3, CH), 127.99 (d, J_{P} 12.8, Ar CH), 128.06 (d, J_{P} 12.5, Ar CH), 128.11 (d, J_{P} 13.3, Ar CH), 127.97 (d, J_{P} 123.2, Ar C), 127.88 (d, J_{P} 120.8, Ar C), 127.75 (d, J_{P}

122.3, Ar C), 132.34 (d, J_P 2.9, Ar CH), 132.50 (d, J_P 2.7, Ar CH), 132.44 (d, J_P 3.7, Ar CH), 132.80 (d, J_P 9.8, Ar CH), 132.67 (d, J_P 9.9, Ar CH), 132.65 (d, J_P 9.4, Ar CH), 172.32 (CCO₂), 172.80 (CCO₂), 172.89 (CCO₂).

LRMS m/z (ESI) 457.4 [M+H]⁺.

HRMS (ESI) calcd. for C₂₃H₄₂O₅PSi 457.2534, found 457.2537 [M+H]⁺.

H₂N-(S)-Leu-(S)-Leu-OBn 443



TFA (5 ml, 65 mmol) was added to a stirred solution of compound **399** (5.39 g, 12 mmol) in dichloromethane (100 ml) maintained at 0 °C for 1 h. On completion of the reaction the reaction mixture was poured into saturated NaHCO₃ (50 ml) and the organic layer was separated. The organic phase was washed with NaHCO₃ (2 x 25 ml). The combined organic extracts were washed with brine and then dried over Na₂SO₄ and stripped of solvent *in vacuo* to give the title compound (3.36 g, 10 mmol, 81% yield) as a colourless oil.

$\nu_{\max}/\text{cm}^{-1}$ 3195 3058 2870-2957 1672 1585 1513 1443 1412 1385 1367 1321 1260 1234 1173 1149

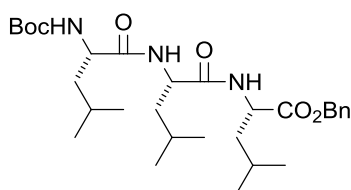
δ_H (600 MHz, CDCl₃) 0.94 (6H, d, J 6.3, CH₃), 0.98 (6H, d, J 6.4, CH₃), 1.57-1.62 (2H, m, CH₂), 1.75-1.84 (4H, m, CH₂ + 2x CHMe₂), 3.94-3.97 (2H, dt, J 2.9, 6.4, 2x NCHCO), 4.69 (2H, s, CH₂Ph), 6.77 (2H, br s, NH₂), 7.26-7.31 (1H, m, Ar H), 7.34-7.37 (4H, m, Ar H).

δ_C (151 MHz, $CDCl_3$) 21.25 (CH_3), 23.40 (CH_3), 24.36 (2x $CHMe_2$), 43.64 (2x CH_2), 53.44 (2 x $NCHCO_2$), 65.48 (CH_2Ar), 127.12 (2x Ar CH), 127.78 (Ar CH), 128.69 (2x Ar CH), 141.02 (Ar C), 169.11 (CON + CCO_2).

LRMS m/z (ESI) 335.2 $[M+H]^+$.

HRMS (ESI) calcd. for $C_{19}H_{31}O_3N_2$ 335.2329, found 335.2333 $[M+H]^+$.

Boc-(S)-Leu-(S)-Leu-(S)-Leu-OBn **444**



DCC (2.79 g, 14 mmol), followed by DMAP (0.17 g, 1 mmol) were added to a stirred solution of compound **443** (4.11 g, 12 mmol) and Boc-(L)-Leu-OH (3.13 g, 14 mmol) in dichloromethane (100 ml) maintained at 0 °C. The mixture was left stirring for 3 h and the reaction monitored by TLC. Once complete the reaction mixture was filtered to remove DHU and then stripped of solvent *in vacuo*. The residue was diluted with dichloromethane and poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na_2SO_4 and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 6:1 - 1:1) to give the title compound (6.5 g, 12 mmol, 96% yield) as a white solid.

mp 142-144 °C.

ν_{max}/cm^{-1} 3276 3069 2871-2957 1715 1636 1536 1455 1387 1366 1249 1162 1046 1018

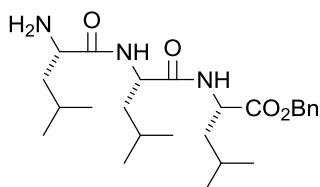
δ_H (400 MHz, $CDCl_3$) 0.85 (6H, d, J 6.5, CH_3), 0.86 (6H, d, J 5.3, CH_3), 0.88 (6H, d, J 6.3, CH_3), 1.41 [9H, s, $C(CH_3)_3$], 1.44-1.49 (2H, m, 2x $CHMe_2$), 1.51-1.62 (6H, m, 3x

CH₂), 1.67-1.69 (1H, m, CHMe₂), 4.48-4.53 (1H, m, CHCO₂), 4.48-4.53 (2H, m, CHCO₂), 5.08 (2H, s, CH₂Ph), 7.29-7.35 (5H, m, Ar H).

δ_C (101 MHz, CDCl₃) 21.35 (CH₃), 21.83 (CH₃), 22.05 (CH₃), 22.31 (CH₃), 22.89 (CH₃), 22.95 (CH₃), 24.36 (2x CHMe₂), 28.40 [C(CH₃)₃], 40.49 (CH₂), 40.97 (CH₂), 41.37 (CH₂), 50.84 (CHCO), 51.47 (CHCO), 51.87 (CHCO₂), 66.94 (CH₂Ar), 80.16 (OCMe₃), 128.28 (2x Ar CH), 128.30 (Ar CH), 128.62 (2 x Ar CH), 135.69 (Ar C), 155.42 (NCO₂), 171.94 (CON), 172.61 (CON), 172.91 (CCO₂).

LRMS m/z (ESI) 570.3 [M+Na]⁺.

H₂N-(S)-Leu-(S)-Leu-(S)-Leu-OBn **445**



TFA (5 ml, 65 mmol) was added to a stirred solution of compound **444** (1.9 g, 3 mmol) in dichloromethane (50 ml) maintained at 0 °C for 1 h. On completion (TLC) of the reaction the reaction mixture was poured into saturated NaHCO₃ (50 ml) and the organic layer was separated. The organic phase was washed with NaHCO₃ (2 x 25 ml). The combined organic extracts were washed with brine and then dried over Na₂SO₄ and stripped of solvent *in vacuo* to give the title compound (1.54 g, 3 mmol, quant. yield) as a colourless oil.

$\nu_{\max}/\text{cm}^{-1}$ 3280 3068 2870-2955 1742 1641 1545 1467 1455 1385 1367 1253 1153

δ_H (600 MHz, CDCl₃) 0.86 (3H, d, J 7.0, CH₃), 0.87 (3H, d, J 6.3, CH₃), 0.88 (3H, d, J 6.6, CH₃), 0.90 (3H, d, J 6.4, CH₃), 0.91 (3H, d, J 6.3, CH₃), 0.94 (3H, d, J 6.34, CH₃), 1.29-1.34 (1H, m, γ CH₂ⁱPr), 1.50-1.56 (2H, m, 2x $\alpha\beta$ CH₂ⁱPr), 1.57-1.73 (1H, m, 3x CHMe₂ + 3x CH₂ⁱPr), 3.36 (1H, dd, J 3.8, 9.9, γ NH₂CH), 4.40-4.44 (1H, m, β NHCH),

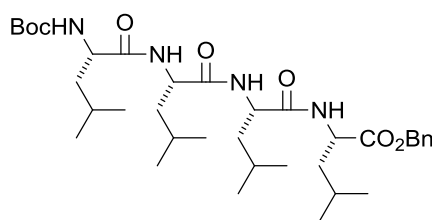
4.55-4.59 (1H, m, $^{\beta}\text{NHCH}$), 5.13 (2H, 2x d, J 12.3, CH_2Ph), 6.70 (1H, d, J 7.6, $^{\alpha}\text{NH}$), 7.30-7.36 (5H, m, Ar H), 7.64 (1H, d, J 8.4, $^{\beta}\text{NH}$).

δ_{C} (151 MHz, CDCl_3) 21.42 (CH_3), 22.02 (CH_3), 22.17 (CH_3), 22.88 (CH_3), 23.01 (CH_3), 23.53 (CH_3), 24.85 (CHMe_2), 24.92 (CHMe_2), 24.96 (CHMe_2), 40.65 (CH_2), 41.30 (CH_2), 44.01 (CH_2), 51.05 (NHCO), 51.24 (NHCO), 53.52 (CHCO_2), 67.14 (CH_2Ar), 127.09 (Ar C), 128.35 (2x Ar CH), 128.49 (Ar CH), 128.70 (2x Ar CH), 135.51 (Ar C), 172.02 (CON), 172.60 (CON), 175.99 (CCO_2).

LRMS m/z (ESI) 470.5 $[\text{M}+\text{Na}]^+$, 448.4 $[\text{M}+\text{H}]^+$.

HRMS (ESI) calcd. for $\text{C}_{25}\text{H}_{42}\text{O}_4\text{N}_3$ 448.3170, found 448.3172 $[\text{M}+\text{H}]^+$.

Boc-(S)-Leu-(S)-Leu-(S)-Leu-(S)-Leu-OBn 446



DCC (1.74 g, 8.44 mmol), followed by DMAP (0.10 g, 0.844 mmol) were added to a stirred solution of Boc-(L)-Leu-OH (1.95 g, 8.44 mmol) and compound **445** (3.44 g, 7.68 mmol) in dichloromethane (100 ml) maintained at 0 °C. The mixture was left stirring for 3 h and the reaction monitored by TLC. The reaction mixture was filtered to remove DHU and then stripped of solvent *in vacuo*. The residue was diluted with dichloromethane and poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na_2SO_4 and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 6:1 - 2:1) to give the title compound (2.01 g, 3.04 mmol, 40% yield) as a colourless oil.

$\nu_{\max}/\text{cm}^{-1}$ 3237 3104 2854-2934 1768 1702 1647 1549 1431 1387 1370 1349 1320 1260
1208 1166 1102

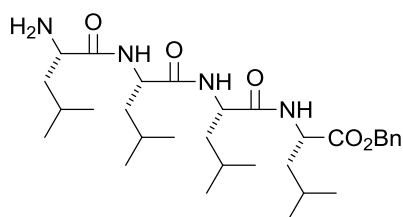
δ_{H} (400 MHz, CDCl_3) 0.83 (6H, d, J 6.5, CH_3), 0.87 (6H, d, J 5.3, CH_3), 0.88 (6H, d, J 6.2, CH_3), 0.90 (6H, d, J 6.4, CH_3), 1.43 [9H, s, $\text{C}(\text{CH}_3)_3$], 1.40-1.51 (3H, m, 3x CHMe_2), 1.53-1.63 (8H, m, 4x CH_2), 1.64-1.68 (1H, m, CHMe_2), 4.36-4.50 (2H, m, 2x CHCH_2), 4.52-4.57 (2H, m, 2x CHCH_2), 5.11 (2H, s, CH_2Ph), 7.27-7.35 (5H, m, Ar H).

δ_{C} (151 MHz, CDCl_3) 21.35 (CH_3), 21.43 (CH_3), 21.56 (CH_3), 22.05 (CH_3), 22.30 (CH_3), 22.70 (CH_3), 22.82 (CH_3), 23.07 (CH_3), 24.36 (CHMe_2), 24.80 (CHMe_2), 25.15 (CHMe_2), 25.22 (CHMe_2), 40.18 (CH_2), 40.49 (CH_2), 40.99 (CH_2), 41.83 (CH_2), 50.85 (NHCO), 51.45 (NHCO), 51.87 (NHCO), 55.46 (CHCO_2), 66.86 (CH_2Ar), 127.22 (Ar C), 128.28 (Ar CH), 128.65 (Ar CH), 128.75 (Ar CH), 135.64 (Ar C), 155.32 (NCO_2), 171.85 (CON), 172.65 (CON), 172.85 (CON), 174.40 (CCO_2).

LRMS m/z (ESI) 683.7 $[\text{M}+\text{Na}]^+$, 661.7 $[\text{M}+\text{H}]^+$.

HRMS (ESI) calcd. for $\text{C}_{36}\text{H}_{64}\text{O}_7\text{N}_5$ 678.4800, found 678.4808 $[\text{M}+\text{NH}_4]^+$.

$\text{H}_2\text{N}-(\text{S})\text{-Leu}-(\text{S})\text{-Leu}-(\text{S})\text{-Leu}-(\text{S})\text{-Leu-OBn}$ **447**



TFA (3 ml, 39 mmol) was added to a stirred solution of compound **446** (2.0 g, 3 mmol) in dichloromethane (75 ml) maintained at 0 °C for 1 h. On completion of the reaction (TLC) the reaction mixture was poured into saturated NaHCO_3 (50 ml) and the organic layer was extracted with dichloromethane (2 x 25 ml). The organic phase was washed with NaHCO_3 (2 x 25 ml). The combined organic extracts were washed with brine and

then dried over Na₂SO₄ and stripped of solvent *in vacuo* to give the title compound (1.38 g, 2 mmol, 82% yield) as a colourless oil.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3270 3068 2860-2958 1735 1540 1460 1450 1380 1370 1257 1150

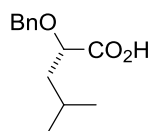
δ_{H} (600 MHz, CDCl₃) 0.80-0.95 (24H, m, 8x CH₃), 1.45-1.57 (2H, m, CH₂ⁱPr), 1.62-1.64 (4H, m, 2x CH₂ⁱPr), 1.67-1.73 (2H, m, 2x CHMe₂), 1.74-1.85 (4H, m, 2x CHMe₂ + CH₂ⁱPr), 3.83-3.89 (1H, m, NH₂CH), 3.92-3.95 (1H, m, NHCH), 4.12-4.16 (1H, m, NHCH), 4.54-4.61 (1H, m, NHCH), 5.10 (2H, 2x d, *J* 12.33, CH₂Ph), 7.28-7.35 (5H, m, Ar H).

δ_{C} (151 MHz, CDCl₃) 21.70 (CH₃), 21.72 (CH₃), 21.82 (CH₃), 22.19 (CH₃), 22.31 (CH₃), 22.72 (CH₃), 22.80 (CH₃), 23.19 (CH₃), 24.39 (CHMe₂), 24.85 (CHMe₂), 25.11 (CHMe₂), 25.19 (CHMe₂), 40.14 (CH₂), 41.01 (CH₂), 41.11 (CH₂), 45.30 (CH₂), 50.95 (NCHCO), 51.15(NCHCO), 51.47 (NCHCO), 55.30 (CHCO₂), 67.22 (CH₂Ar), 127.15 (Ar C), 128.23 (2x Ar CH), 128.52 (Ar CH), 128.70 (2x Ar CH), 135.37 (Ar C), 171.73 (CON), 172.46 (CON), 172.81(CON), 174.54 (CCO₂).

LRMS *m/z* (ESI) 561.5 [M+H]⁺.

HRMS (ESI) calcd. for C₃₁H₅₃O₅N₄ 561.4010, found 561.4015 [M+H]⁺.

(S)-2-Benzyloxy-4-methyl-pentanoic acid **448**



Sodium hydride (1.77 g, 76 mmol) was added to a stirred solution of compound **416** (2.43 g, 19 mmol) in DMF heated to 60 °C for 1 h. The reaction was cooled to 0 °C, benzyl bromide (4.37 ml, 37 mmol) was added slowly and the reaction mixture was again heated at 60 °C for a further 3 h. The solvent was removed *in vacuo* and the

residue was diluted with dichloromethane and poured into water (50 ml) and the organic layer was extracted with dichloromethane (2 x 25 ml). The combined organic extracts were washed with saturated NaHCO₃ then dried over Na₂SO₄ and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 6:1 - 1:1) to give the title compound (3.12 g, 14 mmol, 76% yield) as a colourless oil.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3032 2855-2955 1722 1603 1497 1453 1367 1314 1269 1175 1098 1070 1026

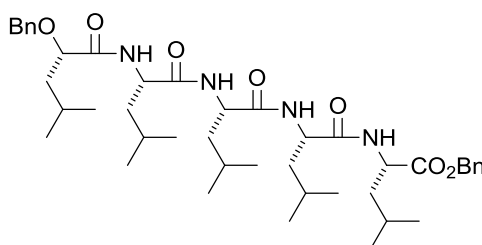
δ_{H} (400 MHz, CDCl₃) 0.85 (3H, d, *J* 6.6, CH₃), 0.93 (3H, d, *J* 6.6, CH₃), 1.50-1.62 (1H, m, CH₂), 1.74-1.80 (1H, m, CH₂), 1.83-1.91 (1H, m, CHMe₂), 4.04 (1H, dd, *J* 4.1, 9.5, CHCO₂), 4.59 (2H, s, CH₂Ph), 7.31-7.35 (5H, m, Ar H).

δ_{C} (101 MHz, CDCl₃) 21.50 (CH₃), 23.18 (CH₃), 24.40 (CHMe₂), 41.79 (CH₂), 72.12 (CH₂Ph), 76.67 (CH), 127.67 (Ar CH), 127.82 (2x Ar CH), 128.43 (2x Ar CH), 129.73 (Ar C), 173.28 (CCO₂).

LRMS *m/z* (ESI) 221.1 [M+H]⁺.

HRMS (ESI) calcd. for C₁₃H₁₇O₃ 221.1183, found 221.1183 [M+H]⁺.

(S)-2-((S)-2-((S)-2-[(S)-2-((S)-2-Benzoyloxy-4-methyl-pentanoylamino)-4-methyl-pentanoylamino]-4-methyl-pentanoylamino)-4-methyl-pentanoylamino)-4-methyl-pentanoic acid benzyl ester 449



DCC (0.61 g, 3 mmol), followed by DMAP (0.04 g, 0.3 mmol) were added to a stirred solution of compound **447** (1.38 g, 2.47 mmol) and compound **448** (0.66 g, 3 mmol) in dichloromethane (100 ml) maintained at 0 °C. The mixture was left stirring for 3 h and

the reaction monitored by TLC. Once complete the reaction mixture was filtered to remove DHU and then stripped of solvent *in vacuo*. The residue was diluted with dichloromethane and poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na₂SO₄ and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 4:1 - 1:1) to give the title compound (1.86 g, 2 mmol, 98% yield) as a white solid.

mp 145-148 °C;

$\nu_{\text{max}}/\text{cm}^{-1}$ 3268 2869-2954 1739 1706 1632 1547 1467 1453 1386 1368 1214 1151 1128
1098 1028

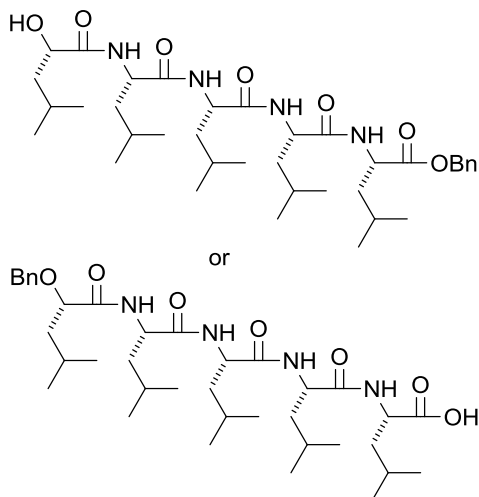
δ_{H} (400 MHz, CDCl₃) 0.75-0.92, 1.0-1.34, 1.36-1.87, 3.76-3.96, 4.28-4.72, 5.02-5.12, 7.24-7.30.

δ_{C} (101 MHz, CDCl₃) 21.50, 21.60, 21.74, 22.08, 22.29, 22.65, 22.93, 23.32, 24.57, 24.76, 24.95, 25.21, 25.60, 25.85, 27.65, 29.30, 29.58, 30.32, 33.31, 33.88, 35.50, 39.83, 40.88, 42.28, 42.36, 47.25, 49.22, 50.65, 50.85, 51.32, 51.47, 55.13, 55.49, 66.93, 72.08, 73.02, 78.93, 136.89, 150.26, 157.06, 161.02, 172.04, 172.44, 173.93, 174.61, 180.28.

LRMS m/z (ESI) 787.7 [M+Na]⁺.

HRMS (ESI) calcd. for C₄₄H₇₂O₇N₅ 782.5426, found 782.5429 [M+NH₄]⁺.

Attempted synthesis of (S)-2-((S)-2-((S)-2-[(S)-2-((S)-2-Hydroxy-4-methylpentanoylamino)-4-methylpentanoylamino]-4-methylpentanoylamino)-4-methylpentanoylamino)-4-methylpentanoic acid **450**



Compound **449** (1.86 g, 2 mmol) and 5% Pd/C (0.8 g, 40% wt) in EtOAc were subjected to hydrogenation conditions overnight. The catalyst was filtered through celite and the solvent was removed *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 4:1 - 1:0) to give the title compound (1.62 g, 2 mmol, 99% yield) as a colourless oil.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3285 2854-2927 1706 1644 1541 1452 1430 1386 1369 1258 1216 1128 1089 1033

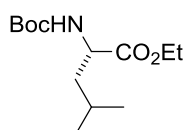
δ_{H} (600 MHz, CDCl_3) 0.81-0.97 (30H, m, 10x CH_3), 1.09-1.22 (9H, m,), 1.25 (10H, s,), 1.28-1.37 (8H, m,), 1.41-1.52 (4H, m,), 1.56-1.77 (19H, m,), 1.81-1.83 (4H, m,), 1.85-1.88 (2H, m,), 1.92-1.94 (3H, m,), 2.08-2.17 (2H, m,), 3.18 (0.5H, s,), 3.43 (1H, t, J 10.47,), 3.88 (1H, tt, J 3.68, 12.30,), 3.95 (1H, dd, J 3.37, 9.43,), 3.99 (0.5H, dd, J 3.99, 9.64,), 4.20 (0.3H, dd, J 3.49, 9.81,), 4.37 (0.5H, d, J 11.95,), 4.42 (1H, d, J 11.28,), 4.47-4.60 (2H, m,), 4.65 (0.3H, d, J 11.88,), 4.73 (0.6H, d, J 11.41,), 5.10 (0.2H, dd, J 3.33, 10.05,), 5.85 (0.7H, d, J 11.52,), 6.65 (0.3H, d, J 6,), 7.29-7.31 (1H, m,), 7.32-7.36 (5H, m,), 8.07-8.09 (0.3H, m,), 8.31 (0.2H, d, J 6.26,).

δ_C (151 MHz, $CDCl_3$) 21.43 21.63 21.78 21.79 22.82 23.19 23.25 23.33 25.00 25.11
25.32 25.68 25.95 29.35 29.41 29.49 29.78 29.82 32.05 33.91 39.92 41.02 41.88 41.97
49.45 51.50 55.27 128.05 128.27 128.54 172.41 173.61 174.62 175.17

LRMS m/z (ESI) 697.5 $[M+Na]^+$.

HRMS (ESI) calcd. for $C_{37}H_{66}O_7N_5$ 692.4957, found 692.4962 $[M+NH_4]^+$.

Boc-(S)-Leu-OEt 451



DCC (7.9 g, 38 mmol), followed by DMAP (0.47 g, 4 mmol) were added to a stirred solution of Boc-(L)-Leu-OH (8.04 g, 35 mmol) and EtOH (2.2 ml, 38 mmol) in dichloromethane (100 ml) maintained at 0 °C. The mixture was left stirring for 3 h and the reaction monitored by TLC. Once complete (TLC) the reaction mixture was filtered to remove DHU and then stripped of solvent *in vacuo*. The residue was diluted with dichloromethane and poured into water (50 ml) and the organic layer was extracted with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na_2SO_4 and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 4:1 - 2:1) to give the title compound (7.86 g, 30 mmol, 87% yield) as a colourless oil.

ν_{max}/cm^{-1} 3322 2852-2929 2117 1714 1625 1568 1531 1449 1390 1366 1346 1310 1271
1243 1160 1088 1046 1024

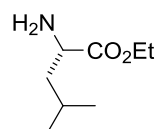
δ_H (400 MHz, $CDCl_3$) 0.95 (3H, d, J 6.5, $\underline{CH_3}$), 0.96 (3H, d, J 6.5, $\underline{CH_3}$), 1.29 (3H, t, J 7.1, $OCH_2\underline{CH_3}$), 1.45 [9H, s, $C(\underline{CH_3})_3$], 1.48-1.53 (2H, m, $\underline{CH_2}^iPr$), 1.64-1.70 (1H, m,

CHMe_2), 4.19 (2H, q, J 7.0, OCH_2CH_3), 4.27-4.33 (1H, m, HNCH), 4.91 (1H, d, J 7.8, NH).

δ_{C} (101 MHz, CDCl_3) 14.18 (OCH_2CH_3), 21.94 (CH_3), 22.84 (CH_3), 24.71 (CHMe_2), 28.32 [$\text{C}(\text{CH}_3)_3$] 41.94 (CH_2), 52.12 (NCHCO), 55.76 (CHCO_2), 61.16 (CH_2), 79.75 (OCMe_3), 156.95 (NCO_2), 173.47 (CCO_2).

LRMS m/z (ESI) 260.2 $[\text{M}+\text{H}]^+$.

$\text{H}_2\text{N}-(\text{S})\text{-Leu-OEt}$ 452



TFA (5 ml, 65 mmol) was added to a stirred solution of compound **451** (3.59 g, 14 mmol) in dichloromethane (75ml) maintained at 0 °C for 1 h. On completion of the reaction (TLC) the reaction mixture was poured into saturated NaHCO_3 (50 ml) and the organic layer was extracted with dichloromethane (2 x 25 ml). The organic phase was washed with NaHCO_3 (2 x 25 ml). The combined organic extracts were washed with brine and then dried over Na_2SO_4 and stripped of solvent *in vacuo* to give the title compound (1.90 g, 12 mmol, 86% yield) as a colourless oil.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3322 2850-2927 1709 1624 1569 1536 1448 1436 1310 1271 1242 1229 1185
1159 1087 1046

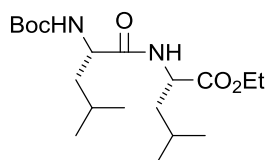
δ_{H} (400 MHz, CDCl_3) 0.86-0.91 (6H, m, 2x CH_3), 1.21 (3H, t, J 8.0, OCH_2CH_3), 1.51-1.56 (2H, m, CH_2), 1.60-1.64 (1H, m, CHMe_2), 3.79-3.89 (1H, m, H_2NCH), 4.11 (2H, q, J 11.0, OCH_2CH_3).

δ_{C} (101 MHz, CDCl_3) 16.71 (OCH_2CH_3), 21.62 (CH_3), 21.95 (CH_3), 25.85 (CHMe_2), 40.92 (CH_2), 51.38 (CHCO_2), 61.34 (OCH_2), 172.63 (CCO_2).

LRMS m/z (ESI) 160.2 $[M+H]^+$.

HRMS (ESI) calcd. for $C_8H_{17}O_2N$ 160.1330, found 160.1332 $[M+H]^+$.

Boc-(S)-Leu-(S)-Leu-OEt **453**



DCC (4.5 g, 21.7 mmol), followed by DMAP (0.27 g, 2.17 mmol) were added to a stirred solution of compound **452** (3.14 g, 19.7 mmol) and Boc-(L)-Leu-OH (5.0 g, 21.7 mmol) in dichloromethane (100 ml) maintained at 0 °C. The mixture was left stirring for 3 h and the reaction monitored by TLC. Once complete (TLC) the reaction mixture was filtered to remove DHU and then stripped of solvent *in vacuo*. The residue was diluted with dichloromethane and poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na_2SO_4 and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 4:1 - 1:1) to give the title compound (7.1 g, 19 mmol, 96% yield) as a white solid.

mp 134-136°C.

ν_{max}/cm^{-1} 3356 3263 3084 2870-2959 1754 1685 1654 1558 1514 1466 1450 1391 1365
1318 1294 1271 1227 1250 1153 1044 1016

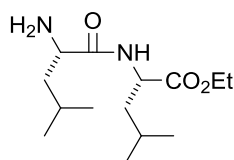
δ_H (400 MHz, $CDCl_3$) 0.85-0.89 (12H, m, 4x $\underline{CH_3}$), 1.20 (3H, t, J 7.2, $OCH_2\underline{CH_3}$), 1.38 [9H, s, $C(\underline{CH_3})_3$], 1.44-1.52 (2H, m, $\underline{CH_2}^iPr$), 1.54-1.67 (4H, m, 2x $\underline{CH}Me_2$ + $\underline{CH_2}^iPr$), 4.03-4.05 (1H, m, $NH\underline{CH}$), 4.11 (2H, q, J 7.1, $OCH_2\underline{CH_3}$), 4.49-4.55 (1H, td, J 4.8, 8.9, $NH\underline{CH}$), 4.87-4.89 (1H, m, NH), 6.39 (1H, d, J 8.2, NH).

δ_C (101 MHz, $CDCl_3$) 14.14 (OCH_2CH_3), 21.83 (CH_3), 22.16 (CH_3), 22.85 (CH_3), 22.88 (CH_3), 24.66 ($CHMe_2$), 24.88 ($CHMe_2$), 28.27 [$C(CH_3)_3$], 40.88 (CH_2), 41.54 (CH_2), 50.70 ($NCHCO$), 52.97 ($CHCO_2$), 61.34 (CH_2), 80.10 ($OCMe_3$), 155.74 (NCO_2), 172.29 (CON), 172.70 (CCO_2).

LRMS m/z (ESI) 373.4 $[M+H]^+$.

HRMS (ESI) calcd. for $C_{19}H_{40}O_5N_3$ 390.2962, found 390.2961 $[M+NH_4]^+$.

H₂N-(S)-Leu-(S)-Leu-OEt 454



TFA (10 ml, 131 mmol) was added to a stirred solution of compound **453** (7.22 g, 19 mmol) in dichloromethane (100 ml) maintained at 0 °C for 1 h. On completion of the reaction the reaction mixture was poured into saturated $NaHCO_3$ (50 ml) and the organic layer was separated. The organic phase was washed with $NaHCO_3$ (2 x 25 ml). The combined organic extracts were washed with brine and then dried over Na_2SO_4 and stripped of solvent *in vacuo* to give the title compound (3.73 g, 14 mmol, 71% yield) as a colourless oil.

ν_{max}/cm^{-1} 3194 2870-2956 1665 1553 1452 1387 1369 1347 1324 1169 1094 1033

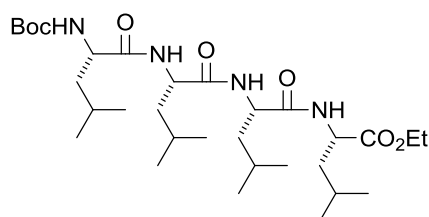
δ_H (400 MHz, $CDCl_3$) 0.94 (6H, d, J 6.4, CH_3), 0.98 (6H, d, J 6.4, CH_3), 1.20 (3H, t, J 7.0, OCH_2CH_3), 1.58-1.63 (3H, m, $CHMe_2$ + CH_2^iPr), 1.75-1.85 (4H, m, $CHMe_2$ + CH_2^iPr + amide NH), 3.95-3.96 (1H, m, $CHCH_2$), 3.97-3.98 (1H, m, $CHCH_2$), 4.10 (2H, q, J 7.1, OCH_2CH_3), 6.73 (2H, s, NH_2).

δ_C (101 MHz, $CDCl_3$) 16.24 (OCH_2CH_3), 21.24 (CH_3), 23.40 (CH_3), 24.37 (2 x $CHMe_2$), 43.61 (2x CH_2), 53.45 ($NCHCO + CHCO_2$), 61.34 (OCH_2), 169.05 ($CON + CCO_2$).

LRMS m/z (ESI) 273.1 $[M+H]^+$.

HRMS (ESI) calcd. for $C_{14}H_{29}O_3N_2$ 273.2173, found 273.2173 $[M+H]^+$.

Boc-(S)-Leu-(S)-Leu-(S)-Leu-(S)-Leu-OEt 455



DCC (1.07 g, 5 mmol), followed by DMAP (0.06 g, 0.5 mmol) were added to a stirred solution of compound **400** (1.78 g, 5 mmol) and compound **454** (1.39 g, 5.17 mmol) in dichloromethane (100 ml) maintained at 0 °C. The mixture was left stirring for 3 h and the reaction monitored by TLC. Once complete (TLC) the reaction mixture was filtered to remove DHU and then stripped of solvent *in vacuo*. The residue was diluted with dichloromethane and poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na_2SO_4 and stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 5:1 - 1:1) to give the title compound (3 g, 5 mmol, 98% yield) as a white solid.

mp 164-167 °C;

ν_{max}/cm^{-1} 3310 2871-2957 1718 1634 1544 1468 1387 1366 1250 1162 1121 1044 1032

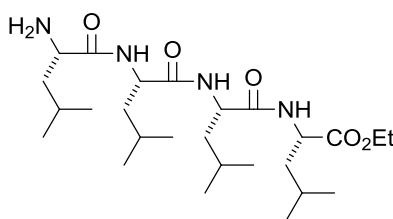
δ_H (600 MHz, $CDCl_3$) 0.82-0.89 (24H, m, 8x CH_3), 1.21 (3H, t, J 6.5, OCH_2CH_3), 1.38 [9H, s, $C(CH_3)_3$], 1.41-1.54 (4H, m, 2x CH_2^iPr), 1.57-1.61 (6H, m, 2x $CHMe_2 + 2x$

CH_2^iPr), 1.63-1.74 (2H, m, 2x CHMe_2), 4.09-4.17 (2H, m, OCH_2CH_3), 4.49-4.54 (2H, m, CHCH_2), 4.60-4.66 (2H, m, CHCH_2).

δ_{C} (151 MHz, CDCl_3) 14.21 (OCH_2CH_3), 21.86 (CH_3), 22.10 (CH_3), 22.43 (CH_3), 22.54 (CH_3), 22.72 (CH_3), 22.81 (CH_3), 22.92 (CH_3), 23.07 (CH_3), 24.75 (CHMe_2), 24.78 (CHMe_2), 24.82 (CHMe_2), 24.87 (CHMe_2), 28.45 (CH_3), 40.90 (CH_2), 41.05 (CH_2), 41.68 (CH_2), 41.82 (CH_2), 50.57 (NHCO_2), 50.84 (NHCO), 51.51 (NHCO), 51.56 (NHCO), 51.76 (CHCO_2), 61.21 (OCH_2CH_3), 79.81 (OCMe_3), 155.99 (NCO_2), 171.99 (CON), 172.10 (CON), 172.81 (CON), 172.96 (CCO_2).

LRMS m/z (ESI) 599.4 $[\text{M}+\text{H}]^+$.

$\text{H}_2\text{N}-(\text{S})\text{-Leu}-(\text{S})\text{-Leu}-(\text{S})\text{-Leu}-(\text{S})\text{-Leu-OEt}$ 456



TFA (5 ml, 65 mmol) was added to a stirred solution of compound **455** (2.05 g, 3 mmol) in dichloromethane (75 ml) maintained at 0 °C for 1 h. On completion of the reaction the reaction mixture was poured into saturated NaHCO_3 (50 ml) and the organic layer was separated. The organic phase was washed with NaHCO_3 (2 x 25 ml). The combined organic extracts were washed with brine and then dried over Na_2SO_4 and stripped of solvent *in vacuo* to give the title compound (1.28 g, 2.57 mmol, 75% yield) as a white solid.

mp 108-111 °C;

$\nu_{\text{max}}/\text{cm}^{-1}$ 3277 3076 2871-2956 1740 1635 1544 1467 1386 1368 1243 1196 1158 1093 1032

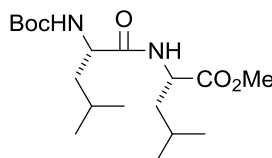
δ_H (600 MHz, $CDCl_3$) 0.78-0.90 (24H, m, 8x $\underline{CH_3}$), 1.22 (3H, t, J 7.2, $OCH_2\underline{CH_3}$), 1.29-1.33 (1H, m, $\underline{CH_2}$), 1.41-1.48 (1H, m, $\underline{CH_2}$), 1.49-1.54 (3H, m, 2x $\underline{CH_2}^iPr$), 1.55-1.61 (5H, m, 2x $\underline{CHMe_2}$ + 2x $\underline{CH_2}^iPr$), 1.63-1.68 (2H, m, 2x $\underline{CHMe_2}$), 3.37-3.40 (1H, m, $\underline{CHCH_2}$), 4.09-4.16 (2H, m, $O\underline{CH_2}CH_3$), 4.42-4.49 (1H, m, $\underline{CHCH_2}$), 4.50-4.55 (2H, m, 2x $\underline{CHCH_2}$).

δ_C (151 MHz, $CDCl_3$) 14.22 (OCH_2CH_3), 21.62 (CH_3), 21.98 (CH_3), 22.34 (CH_3), 22.56 (CH_3), 22.76 (CH_3), 22.95 (2x CH_3), 23.41 (CH_3), 24.78 ($CHMe_2$), 24.87 ($CHMe_2$), 24.88 ($CHMe_2$), 24.89 ($CHMe_2$), 40.98 (CH_2), 41.30 (CH_2), 41.33 (CH_2), 43.95 (CH_2), 50.86 (NHCO), 51.29 (NHCO), 51.70 (NHCO), 53.58 ($CHCO_2$), 61.31 (OCH_2CH_3), 171.82 (2x NCO), 172.29 (CON), 172.83 (CCO_2).

LRMS m/z (ESI) 521.5 $[M+Na]^+$, 499.5 $[M+H]^+$.

HRMS (ESI) calcd. for $C_{26}H_{51}O_5N_4$ 499.3854, found 499.3856 $[M+H]^+$.

Boc-(S)-Leu-(S)-Leu-OMe **461**



DCC (6.98 g, 34 mmol), followed by DMAP (0.413 g, 3 mmol) were added to a stirred solution of compound **460** (4.47 g, 31 mmol) and Boc-(L)-Leu-OH (7.83 g, 34 mmol) in acetonitrile (75 ml) / tetrahydrofuran (75 ml) maintained at 0 °C. The mixture was left stirring for 3 h and the reaction monitored by TLC. The reaction mixture was filtered to remove DHU and then stripped of solvent *in vacuo*. The residue was diluted with dichloromethane and poured into water (50 ml) and the organic layer was separated. The aqueous phase was washed with dichloromethane (2 x 25 ml). The combined organic extracts were dried over Na_2SO_4 and stripped of solvent *in vacuo*. The residue

was subjected to chromatography (EtOAc : Pet. Ether 4:1 - 1:0) to give the title compound (8.27 g, 23 mmol, 75% yield) as a colourless oil.

$\nu_{\text{max}}/\text{cm}^{-1}$ 3348 3267 3088 2870-2956 1759 1683 1654 1560 1516 1464 1436 1364 1273
1250 1198 1153 1121 1096 1044 1029

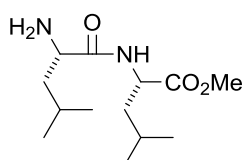
δ_{H} (600 MHz, CDCl_3) 0.84-0.88 (12H, m, CH_3), 1.37 [9H, s, $[\text{C}(\text{CH}_3)_3]$], 1.45-1.54 (2H, m, CH_2), 1.56-1.63 (4H, m, 2x CHMe_2 + CH_2), 3.65 (3H, s, OCH_3), 4.03-4.06 (1H, m, CHMe_2), 4.54 (1H, td, J 4.8, 8.8, CH), 4.87 (1H, d, J 7.7, NH), 6.45 (1H, d, J 7.7, NH).

δ_{C} (151 MHz, CDCl_3) 21.78 (CH_3), 22.13 (CH_3), 22.85 (2x CH_3), 24.64 (CHMe_2), 24.69 (CHMe_2), 28.26 [$\text{C}(\text{CH}_3)_3$], 41.47 (2x CH_2), 50.57 (NHCO), 52.27 (NHCO), 52.89 (CHCO_2), 80.03 (OCMe_3), 155.72 (NCO_2), 172.26 (CON), 173.19 (CCO_2).

LRMS m/z (ESI) 381.4 $[\text{M}+\text{Na}]^+$, 359.5 $[\text{M}+\text{H}]^+$.

HRMS (ESI) calcd. for $\text{C}_{18}\text{H}_{35}\text{O}_5\text{N}_2$ 359.2540, found 359.2541 $[\text{M}+\text{H}]^+$.

$\text{H}_2\text{N}(\text{S})\text{-Leu}(\text{S})\text{-Leu-OMe}$ 458



Trifluoroacetic acid (5 ml, 65 mmol) was added to a stirred solution of compound **461** (1.4286 g, 4 mmol) in dichloromethane maintained at 0 °C. The reaction was monitored by TLC until completion. The solution was poured into saturated NaHCO_3 (50 ml) and the organic layer was separated. The organic extracts were washed with water (2 x 25 ml) and dried using Na_2SO_4 . The combined organic extracts were stripped of solvent *in vacuo*. The residue was subjected to chromatography (EtOAc : Pet. Ether 4:1 - 1:0) to give the title compound (1 g, 3.87 mmol, 97% yield) as a white solid.

mp 253-255 °C;

$\nu_{\text{max}}/\text{cm}^{-1}$ 2871-2957 2319 1648 1455 1388 1369 1348 1327 1266 1227 1171 1137 1120
1034

δ_{H} (600 MHz, CDCl_3) 0.94 (6H, d, J 6.6, CH_3), 0.96 (6H, d, J 6.6, CH_3), 1.57-1.62 (2H, ddd, J 5.3, 9.2, 13.8, CH_2), 1.67-1.71 (2H, ddd, J 4.6, 8.9, 13.6, CH_2), 1.79-1.86 (2H, m, CHMe_2), 3.87-3.89 (2H, dd, J 4.6, 9.1, CH).

δ_{C} (151 MHz, CDCl_3) 20.61 (2x CH_3), 22.31 (2x CH_3), 24.03 (2x CHMe_2), 44.59 (NHCO), 53.37 (CHCO_2), 169.92 ($\text{CON} + \text{CCO}_2$).

LRMS m/z (ESI) 281.4 $[\text{M}+\text{Na}]^+$.

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